Understanding How Pasture Irrigation Influences Soil Nitrous Oxide Fluxes and Nitrous Oxide Reductase

---

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Jen Owens

Lincoln University

2016

Abstract of a thesis submitted in partial fulfilment of the

requirements for the Degree of PhD Soils and Physical Sciences.

Understanding how pasture irrigation influences soil nitrous oxide fluxes and nitrous oxide reductase

by

Jen Owens

This thesis is a combination of field and laboratory studies aimed at understanding how irrigation influences nitrous oxide (N_2O) emissions from grazed pastures in New Zealand. The general goal was to understand how nitrous oxide reductase (N_2OR) was affected by irrigation practices, and other factors, and to assess the potential to minimize N_2O emissions by encouraging N_2O reduction to dinitrogen (N_2).

EXPERIMENT 1 (Chapter 4) - A field monitoring campaign measured N_2O fluxes for 35 days from urine and non-urine treated grazed and irrigated dairy pasture, situated on a free-draining soil. Two irrigation frequencies - a 3 day irrigation frequency and a 6 day irrigation frequency - applied the same total amount of water by the end of the experiment. The original hypothesis was that a more frequent irrigation regime would keep soil moisture higher, thereby lowering soil oxygen (O_2), resulting in greater N_2OR activity, and reduced N_2O emissions. Soil O_2 measurements showed that soil O_2 was lower at 50 and 100 mm soil depth in the more frequently irrigated soil. Denitrification potential measurements taken over the course of the experiment, using the acetylene inhibition method, showed that potential $N_2O/(N_2O+N_2)$ was lower under the more frequent irrigation regime, suggesting greater potential for N_2OR. Contrary to the original hypothesis, however, there was no difference in the N_2O fluxes from the different irrigation frequencies, despite the soil chemical and biological differences. Estimates of soil relative gas diffusivity (D_P/D_O) showed that D_P/D_O was too high for N_2O to be reduced to N_2, according to the thresholds identified by Balaine et al. (2013).

The results from this experiment raised questions warranting exploration.

EXPERIMENT 2 (Chapter 5) Can we further explore and compare how well soil O_2 measurements and D_P/D_O an expression of soil O_2 diffusion, explain N_2O fluxes under variable hydrological conditions on a heavy soil?

EXPERIMENT 3 (Chapter 6) Temporal dynamics of N_2OR and denitrification potential after a wetting event need to be better understood. Can we interpret whether lower $N_2O/(N_2O+N_2)$ is attributed to just increased soil moisture, or is it also related to priming of the microbial pathway for N_2OR?

EXPERIMENT 4 (Chapter 7) Is the diel cycling of soil O_2 temperature and respiration driven? Is there also diel cycling of N_2O and N_2OR related to plant dynamics, such as expulsion of root exudates? Can we isolate and explore these factors by measuring N_2O and $^{15}N\text{-}N_2O$ and $^{15}N\text{-}N_2$ recovery from soils with and without plants in the absence of temperature change?

EXPERIMENT 5 (Chapter 8) The effects of plants on N_2OR should be assessed by comparing rhizosphere and bulk soils to enable laboratory results, many of which do not include plants, to be transferred to field scenarios, where plants are common. Along this same thread, spatially variability in the field should be explored, as this variability can inform sampling strategies and extrapolation of local results.

Keywords: irrigation frequency, urine, relative soil gas diffusivity, soil oxygen

Acknowledgements

This PhD project has been a great opportunity for me to focus entirely on becoming a better scientist through the mentorship and guidance from a lot of people. For this, I owe a great debt to my supervisory committee; Professor Tim Clough from Lincoln University, Dr. Johannes Laubach from Landcare Research, Dr. John Hunt from Landcare Research, Dr. Rodney Venterea, and Dr. Nik Lehto from Lincoln University. I must give an special thanks to Tim for his countless hours providing feedback and guidence.

I could not have completed my work without technical help from Niel Smith, who is part technician, part guidance councillor. I feel I owe him more than a thank you or a muffin basket. I also received much needed help in the field from Greg Curlin to whom I own a thank you.

Thank you to the people at Lincoln's Analytical Services team. This includes Lynne Cluase, Leanne Hassall, Qian Liang, Barry Anderson, Vicky Zhang, and an espeically to Roger Creswell and Manjula Premaratne for "making mountains move" to help me when needed, whether it be bumping me to the front of the line to get my samples run, or fulfilling my more obscure requests.

Thank you to Keith Cameron and the Centre for Soil and Environmental Quality for generously loaning equipment and space, and a special thank you to Nigel Beale, Carol Barlow, and Stephen Moore, all of whom have lended hands, wise counsel, and sympathetic ears during my time here.

I would also like to thank Kieth Cameron, Di Hong, and Janet Berterm for giving me to the opportunity to work part-time.

I wish to also express my gratitude to Landcare Research for their financial and practical support. A special thank you to Anitra Fraser, Graeme Rogers, and Dr. Rebecca Phillips for their valuable assistance in the field and lab, and for all their input which helped make my research better.

Thank you to Caitriona Cameron and Dean O'Connell at the library; Caitriona for her efforts helping to improve my skills at the post-graduate conferences, Thr3sis, and workshops, and Dean for his help with statistics.

Lastly, thank you to the friends I've made here at Lincoln. I won't name you all because Lincoln is a place of transients and I have met so many people over the last three years. If you're reading this, thank you for the support, good times, and memories. You should get back to work now. But I feel like my office mates Wei, and then Nimlesh, deserve a special mention. Thank you to you both for the conversation, feedback, company and friendship.

Part of this thesis has been accepted and submitted for publication. These are:

Chapter 4 - Accepted to the Journal of Environmental Quality (doi:10.2134/jeq2015.10.0516)

Nitrous Oxide Fluxes, Soil Oxygen, and Denitrification Potential of Urine- and Non-Urine-Treated Soil under Different Irrigation Frequencies

Jen Owens, Tim J. Clough, Johannes Laubach, John E. Hunt, Rodney T. Venterea and Rebecca L. Phillips

Chapter 5 - Submitted to the Soil Science Society of America Journal (manuscript ID S-2016-09-0277-OR)

Nitrous oxide fluxes and soil oxygen dynamics of soil treated with cow urine

Jen Owens, Tim J. Clough, Johannes Laubach, John E. Hunt, Rodney T. Venterea

Table of Contents

List of Figures

List of Tables

Acronyms and Abbreviations

ρb	Soil bulk density (Mg m^{-3})
ψ	Matric potential (-kPa)
D_P	Gas diffusion coefficient in soil (m^3 soil air m^{-1} soil s^{-1})
D_O	Gas diffusion coefficient in air (m^3 soil air m^{-1} soil s^{-1})
D_P/D_O	Relative gas diffusivity
φ	Total porosity (m^3 air m^{-3} soil)
ε	Air-filled porosity (m^3 air m^{-3} soil)
ρd	Soil particle density (Mg m^{-3})
θg	Gravimetric water content (g water g^{-1} dry soil)
θv	Volumetric water content (m^3 water m^{-3} soil)
AOB	Ammonia oxidising bacteria
AOA	Ammonia oxidising archeae
DI	Deionised water
C	Carbon
N	Nitrogen
HWC	Hot water carbon
CWC	Cold water carbon
GC	Gas chromatograph
N_2O	Nitrous oxide
NO_3-N	Nitrate nitrogen
NO_2^--N	Nitrite nitrogen
NH_4^+-N	Ammonium nitrogen
N_2	Dinitrogen gas
O_2	Oxygen gas
SEM	Standard error of mean
WFPS	Water-filled pore space
SWLR	Structure dependent Water-induced linear reduction

Chapter 1. Introduction

The intergovernmental Panel on Climate Change (IPCC) warns that atmospheric concentrations of greenhouse gases (GHG) have increased since pre-industral times (~1750 AD) and that this increase in GHG's is contributing to global warming (Mosier et al. 1998, Meyer et al. 2014). Atmospheric concentrations of nitrous oxide (N_2O) have increased 20% over this time. Nitrous oxide has a global warming potential of 298 compared to carbon dioxide (CO_2) over a 100 year time period, an atmospheric lifetime of ~121 years and is responsible for 5-6% of all global warming (Mosier et al. 1998, Meyer et al. 2014). Nitrous oxide is also the most important ozone depleting substance currently emitted (Ravishankara et al. 2009). Ozone depletion occurs due to catalytic reactions of nitrogen oxides (NO_x), including N_2O, with ozone in the stratosphere (Ravishankara et al. 2009). Because of the detrimental environmental effects of N_2O, humanity has a stake in identifying sources of N_2O emissions, and coming up with strategies to reduce these emissions.

Increases in atmospheric concentrations of N_2O can be attributed to agricultural soils, which are responsible for 10-12% of global anthropogenic GHG emissions as CO_2 equivalent (Forster et al. 2007). Greenhouse gas emissions from agricultural soils are driven by increased nitrogen (N) inputs (Davidson 2009). There are increasing efforts to understand the effects of land management practices on N_2O fluxes from soils, however, there are still many unknowns.

Irrigation is commonly used to aid crop production (Mosier et al. 1986, Scheer et al. 2012). Irrigation is also used to improve pasture quality for grazing cattle, especially by the dairy industry in New Zealand. Few studies have assessed how irrigation affects N_2O emissions from urine patches. Urine patches are "hot spots" for N_2O production (Clough et al. 1996, Lovell and Jarvis 1996, van Groenigen et al. 2005a, van Groenigen et al. 2005b) and irrigation may provide opportunities to reduce the amount of N_2O emitted. One of the strategies for this involves invoking conditions that are favorable for N_2O reductase (N_2OR) production, which is the enzyme responsible for reducing N_2O to dinitrogen (N_2) (Knowles 1982). Dinitrogen is an inert gas which is a natural constituent of the atmosphere. To pursue strategies to reduce N_2O by encouraging N_2OR activity, there needs to be a more comprehensive understanding of how agricultural management practices (irrigation, grazing) and related factors (urine, plant dynamics) influence soil oxgyen (O_2)

concentrations, since O_2 concentration is a proximal controller of N_2OR activity (Firestone and Davidson 1989).

This thesis focuses on quantifying N_2O fluxes and N_2OR from grazed pasture soils. The main objectives are:

- To evaluate how irrigation frequency influences N_2O and N_2OR potential from urine-treated pasture soil. This work includes measuring soil O_2 under a urine patch and under different irrigation regimes.

- To assess how well soil O_2, hydrological variables, and modeled relative soil gas diffusivity (D_P/D_O) explain N_2O fluxes under variable soil moisture conditions *in situ*.

- To quantify temporal dynamics of N_2OR potential with time since a wetting event, while holding soil moisture constant after the wetting event. This work explores how soil biological and chemical factors change to affect N_2OR by observing them over time without changes in soil moisture.

- To determine if there is a diel cycling of N_2OR in the absence of temperature change. This work compares soils with and without plants to determine whether diel cycling of root exudates from the rhizosphere affects daily N_2OR cycles.

- To assess whether there is a difference in N_2OR potential from bulk and rhizosphere pasture soil.

This thesis is divided into nine chapters. After this introductory chapter, a review of the literature relevant to N_2O and N_2OR is presented in Chapter 2. This includes a summary of the pathways for N_2O production and an overview of N_2OR dynamics, a review of previous work relating soil O_2 to N_2O and N_2OR from pastures, and identification of research gaps. Chapter 3 provides a general overview of the methods used in the experiments, which is referenced in the subseqent chapters.

Chapter 4, Chapter 5, Chapter 6, Chapter 7, and Chapter 8 present study rationales, a brief outline of methods, the results, and discussions for the research conducted during this PhD. Chapters 5 and 6 are presented as manuscripts.

Chapter 4, Experiment 1, focuses on how irrigation frequency influences N_2O, N_2OR, and soil O_2 from urine-treated free draining grazed pasture soil *in situ*. It introduces the concept of relative

soil gas diffusivity (D_P/D_O). This chapter has been published as "Nitrous Oxide Fluxes, Soil Oxygen, and Denitrification Potential of Urine- and Non-Urine Treated Soil Under Different Irrigation Frequencies" in the Journal of Environmental Quaility (doi:10.2134/jeq2015.10.0516).

Experiment 1 was a field trial conducted on an irrigated and grazed dairy paddock. This assessed the influence of two irrigation frequencies (3 day irrigation frequency vs 6 day irrigation frequency) on N_2O fluxes and potential $N_2O/(N_2O+N_2)$ ratios obtained from denitrification enzyme assays (DEA) (hereon called DEA-N_2O/(DEA-N_2O+N_2)) from urine and non-urine treated soils under both irrigation regimes. It was hypothesized that N_2OR would remain active in the soil under a more frequently irrigated moisture regime, leading to lower total N_2O fluxes.

Consistent with the original hypothesis, the DEA-N_2O/(DEA-N_2O+N_2) ratio from the DEA's were lower under the 3 day irrigation treatment compared to the 6 day treatment, suggesting greater potential N_2OR. Soil moisture was also higher under the more frequent irrigation and soil O_2 was lower at 50 and 100 mm soil depths. However, contrary to the original hypothesis, there was no significant difference between N_2O fluxes under different irrigation regimes. These results can be rationalized by considering that soil O_2 concentration data suggest that the soil was well aerated throughout the experiment, which is supported by the D_P/D_O data. Perhaps differences in soil O_2 were more extensive at the micro-pore scale.

Chapter 5, Experiment 2, measured N_2O fluxes from urine and no urine treatments on a heavy, poorly drained soil during hydrologically variable conditions (rain, surface flooding, and heavy irrigation) *in situ*. It compares how well different hydrological variables including water-filled pore space (WFPS) and volumeteric water content (θv), as well as soil O_2 measurements, and modelled D_P/D_O related to measured N_2O fluxes. The paper has been submitted to Soil Science Society of America Journal (manuscript ID S-2016-09-0277-OR)

During this second field trial N_2O fluxes were measured daily for 55 days *in situ* using static chambers. Soil samples were taken every six days over the course of the experiment for ancillary data including inorganic N, organic C, soil pH, and conductivity.

It was hypothesized that N_2O fluxes would be best explained by soil O_2 concentrations or D_P/D_O. The results show that daily average modelled D_P/D_O strongly related to daily N_2O fluxes. There were instances of negative N_2O fluxes at the end of the experiment following sustained wetting

and heavy irrigation, suggesting wet antecedent moisture conditions primed the N_2OR pathway for N_2O uptake.

Chapter 6, Experiment 3, was a laboratory experiment that assessed the temporal dynamics of N_2OR after a wetting event in repacked soil cores held on tension tables. The tenion tables were used to stablize soil moisture during the experiment. This experiment explored how chemical and biological controls on N_2OR were influenced by wet antecedent moisture conditions.

This experiment also served to provide insight into the differences in denitrification potential and DEA-N_2O/(DEA-N_2O+N_2) ratios observed between the two irrigation frequencies during Experiment 1, and the occurrences of negative fluxes during Experiment 2. Denitrification enzyme assays using acetylene inhibition and non-limiting conditions for denitrification (anaerobic with the addition of NO_3^- and C) were used to assess denitrification potential and denitrification end-product ratios over time. Denitrification potential was measured 7 times over 42 days, along with inorganic N, organic C, and soil pH. The effects of differing incubation times during the DEA were also assessed.

The results from this experiment support the original hypothesis that the ratio of DEA-N_2O/(DEA-N_2O+N_2) would be lowest immediately following the wetting event, and would gradually increase with time since the wetting event. This is due to a gradual decline N_2OR activity with time since a wetting event. The O_2-limited conditions imposed by the wetting event encouraged N_2OR production.

This experiment also explored how incubation time during DEA influenced the results. This was done because there are a range of incubation times used in studies. The results found that longer DEA incubation times increased denitrification potential, and also decreased the DEA-N_2O/(DEA-N_2O+N_2) ratios.

The results from Experiment 3 help inform Experiment 1. The results suggest that potential N_2OR was higher under the 3 day irrigation regime compared to the 6 day irrigation regime not only because of higher soil moisture, but also due to sustained N_2OR functioning after a wetting event, which decreased with time since the wetting event. Experiment 3 also helps inform Experiment 2. The results suggest that the episode of N_2O uptake observed at the end of Experiment 2 after heavy irrigation was due to a priming of the N_2OR pathway that occurred because of the earlier flooding event.

Chapter 7, Experiment 4, was a laboratory experiment that aimed to determine if there is a diel cycling effect on N_2OR in the absence of temperature change. The rationale is that diel cycling of plant root exudates could affect N_2OR, along with O_2. There was an apparent daily cycling of soil O_2 observed in Experiment 1 and 2 field trials, likely associated with plant respiration. However, plants not only contribute to variation in soil O_2/CO_2, the rhizosphere can contribute exudates, which may influence N_2OR activity. Deciphering this may help to understand diel cycling of N_2O fluxes. This experiment sought to understand how plants may affect N_2OR and if diel cycling of N_2OR might occur.

It was expected that there would be greater N_2O and N_2 fluxes, and denitrification potential, from soils in the presence of plants compared to soils without plants. It was hypothesized that, if there was diel cycling of N_2OR, it would result in greater N_2 emitted during daylight coinciding with daily plant respiration. The results failed to show consistent diel trends in N_2OR due to plants. This is possibly due to the changes to soil structure created by the rhizosphere in the planted soil cores, which resulted in higher average O_2 in the plant treatment, despite equivalent WFPS/θv in both treatments. There was higher DEA in soils with plants compared to no plants, suggesting more biological potential for denitrification in soil due to the presence of plants.

Chapter 8, Experiment 5, compares N_2OR in bulk soil (not affected by plant roots) and rhzisphere soil (affected by plant roots) collected from six locations on a grazed pasture. The intention was to determine if there was greater N_2OR in the rhizosphere soil compared to the bulk soil, because it was expected that the conditions for N_2OR would be more favorable in the rhizosphere soil.

The hypothesis for this experiment was that the presence of plants would affect the soil environment resulting in higher labile C concentrations due to expulsion of root exudates, and that O_2 depletion would occur due to plant respiration differences in soil labile C availability. Therefore, O_2 depletion would be exaggerated proximate to the plant root compared to soil at a distance from the plant roots. Because of this, it was expected that there would be a greater denitrification potential, N_2O, and N_2 production in the rhizosphere soil, where conditions are more favourable, compared to the bulk soil.

This experiment collected bulk and rhizosphere soil in the field from six different sites in three different paddocks on one farm. Back in the lab, under a 0% O_2 headspace, N_2O and N_2 concentrations were measured for 36 hours from each soil sample. Denitrification potential measurements and soil nutrient extractions were performed using soil from the same sites.

The results failed to show a difference between the bulk and rhizosphere soil for N_2O, N_2, or the ratio of N_2O:N_2. However, there were differences between paddocks, indicative of spatial variability. This could be due to grazing or fertilizer history, or natural spatial variability. More data is needed in future experiments to examine the ideas raised.

Chapter 9 provides an overview of the experiments preformed in this thesis, and concludes with future research suggestions.

The linkage between the experiments is presented in Figure 1.1, which shows the flow of the experimental questions throughout the thesis.

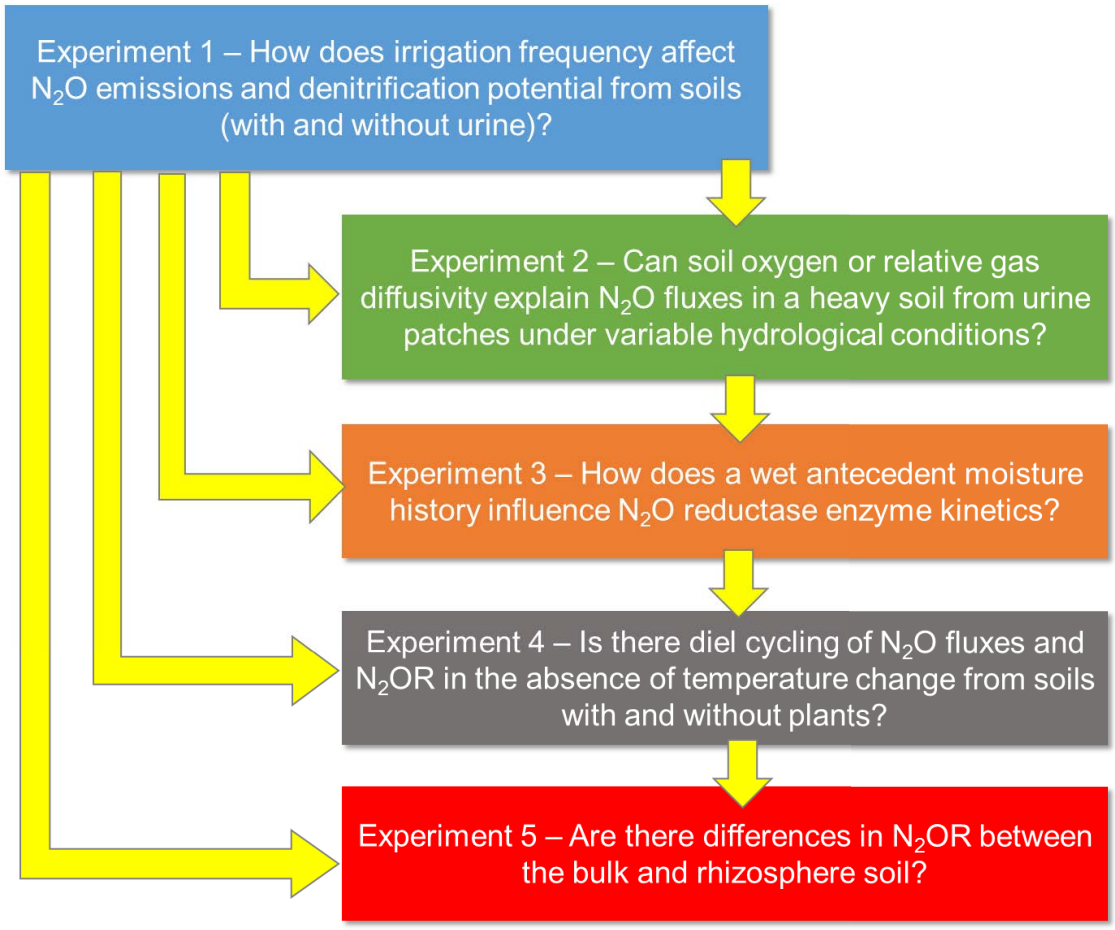


Figure 1.1 A map of the thesis outline, showing the research question for each experiment, and linking the experiments together to show how one informs another.

1.1 References

Clough, T. J., R. R. Sherlock, K. C. Cameron, and S. F. Ledgard. 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant and Soil 178:141-152.

Davidson, E. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nature Geoscience 2:659-662.

Firestone, M., and E. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil.in M. O. Andreae and D. S. Schimel, editors. Exchange of Trace Gases Between Ecosystems and the Atmosphere. Wiley, New York.

Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D. W. Fahey, J. Haywood, J. Lean, D. C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R. Van Dorland. 2007. Changes in atmospheric constituents and in radiative forcing.in S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, Cambridge, UK, and New York.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Lovell, R. D., and S. C. Jarvis. 1996. Effects of urine on soil microbial biomass, methanogenesis, nitrification and denitrification in grassland soils. Plant and Soil 186:265-273.

Meyer, L., S. Brinkman, L. van Kesteren, N. Leprince-Ringuet, and F. van Boxmeer. 2014. IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland.

Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. van Cleemput. 1998. Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. Nutrient Cycling in Agroecosystems 52:225-248.

Mosier, A. R., W. D. Guenzi, and E. E. Schweizer. 1986. Soil Losses of Dinitrogen and Nitrous Oxide from Irrigated Crops in Northeastern Colorado. Soil Science Society of America Journal 50:344-348.

Ravishankara, A. R., J. Daniel, and R. Portmann. 2009. Nitrous Oxide (N_2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. Science 326:123-125.

Scheer, C., P. R. Grace, D. W. Rowlings, and J. Payero. 2012. Nitrous oxide emissions from irrigated wheat in Australia: Impact of irrigation management. Plant and Soil 359:351-362.

van Groenigen, J., P. Kuikman, W. de Groot, and G. Velthof. 2005a. Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. Soil Biology and Biochemistry 37:463-473.

van Groenigen, J. W., G. L. Velthof, F. J. E. van der Bolt, A. Vos, and P. J. Kuikman. 2005b. Seasonal variation in N_2O emissions from urine patches: Effects of urine concentration, soil compaction and dung. Plant and Soil 273:15-27.

Chapter 2. Literature Review

2.1 Significance of Nitrous Oxide Emissions and the Environmental Implications of Increasing Nitrous Oxide Emissions

Agriculture is responsible for 10-12% of all total greenhouse gas (GHG) emissions on a carbon dioxide (CO_2) equivalent basis, and contributes 58% of the total anthropogenic nitrous oxide (N_2O) emissions (Smith et al. 2007). However, in New Zealand (NZ), the agricultural sector is the primary source of GHGs, contributing 48% of the country's total emissions (Ministry for the Environment 2013). Agricultural soils are also the main source of N_2O in NZ, accounting for 95% of the total N_2O emitted (Ministry for the Environment 2013). Upwards of 80% of these N_2O emissions are directly related to livestock excretal returns to the soil (de Klein and Ledgard 2005).

2.1.1 Dairying and Irrigation in New Zealand

In NZ, expansion of the dairy industry has driven the increase in N_2O emissions from 1990 (Ministry for the Environment 2013). The country is home to more than 6 million dairy cows (Statistics Zealand, 2012). Upwards of 55% of NZ land surface is grazed pastures (Ministry for the Environment 2010), consisting mostly of perennial ryegrass (*Lolium perenne L.*) and white clover (*Trifolium repens L.*) (Dodd et al. 2011), commonly grazed year-round (MacLeod and Moller 2006).

Irrigation has been widely implemented to improve pasture quality and yield for grazing dairy cattle (Hopewell 1958, 1960, McBride 1994, Thom et al. 2001). This allows cattle to meet many of their nutritional needs through grazing which improves milk quality and quantity (Thom et al. 2001). An estimated 721,700 ha of NZ's land is irrigated (New Zealand Government National Infrastructure Unit 2015). Most of this is in the South Island, with 562,900 ha of irrigated land located in Canterbury (New Zealand Government National Infrastructure Unit 2015).

There has been little research done to understand how N_2O emissions are affected by irrigation onto grazed pasture. The diversity in irrigation regimes used (i.e. flood vs drip) and the various strategies used to deploy them, make it difficult to extrapolate the results from earlier irrigation studies to NZ irrigated dairy pasture systems. Flood irrigation, or inundation irrigation, has fallen out of favour because it has the potential to invoke soil leaching of nitrate (NO_3^-) or other contaminants (Saunders and Saunders 2012).

Much of the past research related to irrigation has focused on how irrigation regimes influence N_2O fluxes from cropped soils (Mosier et al. 1986, Simojoki and Jaakkola 2000, Liu et al. 2011, Sainju et al. 2012, Scheer et al. 2012, 2013, Maharjan et al. 2014). Few general conclusions can be drawn from these studies because of the diverse range of irrigation strategies used on various soil and vegetation types. The general assumption is that N_2O emissions from irrigated soils are expected to increase due to the positive relationship between soil moisture and N_2O fluxes (Trost et al. 2013). Some studies have reported increases in N_2O fluxes with irrigation (Simojoki and Jaakkola 2000, Sainju et al. 2012) while others have not (Liu et al. 2011, Maharjan et al. 2014). Irrigation frequency has been found to affect N_2O fluxes in some instances, and the effect of irrigation partially depends on other soil conditions e.g. pH (Scheer et al. 2012, 2013). There can be difficulty maintaining differences between irrigation treatments *in situ* when environmental factors, such as precipitation, disrupt treatment effects (Scheer et al. 2013).

Little attention has been given to understanding how irrigation practices will affect N_2O emissions from urine patches. Some inferences about this can be made from studies that have focused on how irrigation affects N_2O fluxes from fertilized soil. Such studies note high N_2O fluxes after fertilization, precipitation, or flood irrigation (Phillips et al. 2007, Sainju et al. 2012). Little is known about how non-flood or sprinkler irrigation affects N_2O from grazed pastures. Urine patches are a known hotspot for N_2O emissions (Clough et al. 2004). Nitrogen (N) substrate type and availability, which differs between fertilizer and ruminant urine, and also differs over time after urine deposition, will influence how N_2O emissions behave under irrigation.

2.1.2 Nitrogen Cycling in Grazed Pastures

Managed grasslands, such as intensively grazed or fertilized grasslands, emit more N_2O per surface area than arable or forested soil (Oenema et al. 1998), making these landscapes important sources for N_2O emissions. A summary of N inputs, losses, and transformations within a pasture system are presented in Figure 2.1. High N concentrations in agricultural soil result from fertilizer application (Bouwman et al. 2002) and urine deposition (Selbie et al. 2014), which can provide substrates for microbial or chemical production of N_2O (Carran and Clough 1996, Mosier et al. 1998, Pathak 1999).

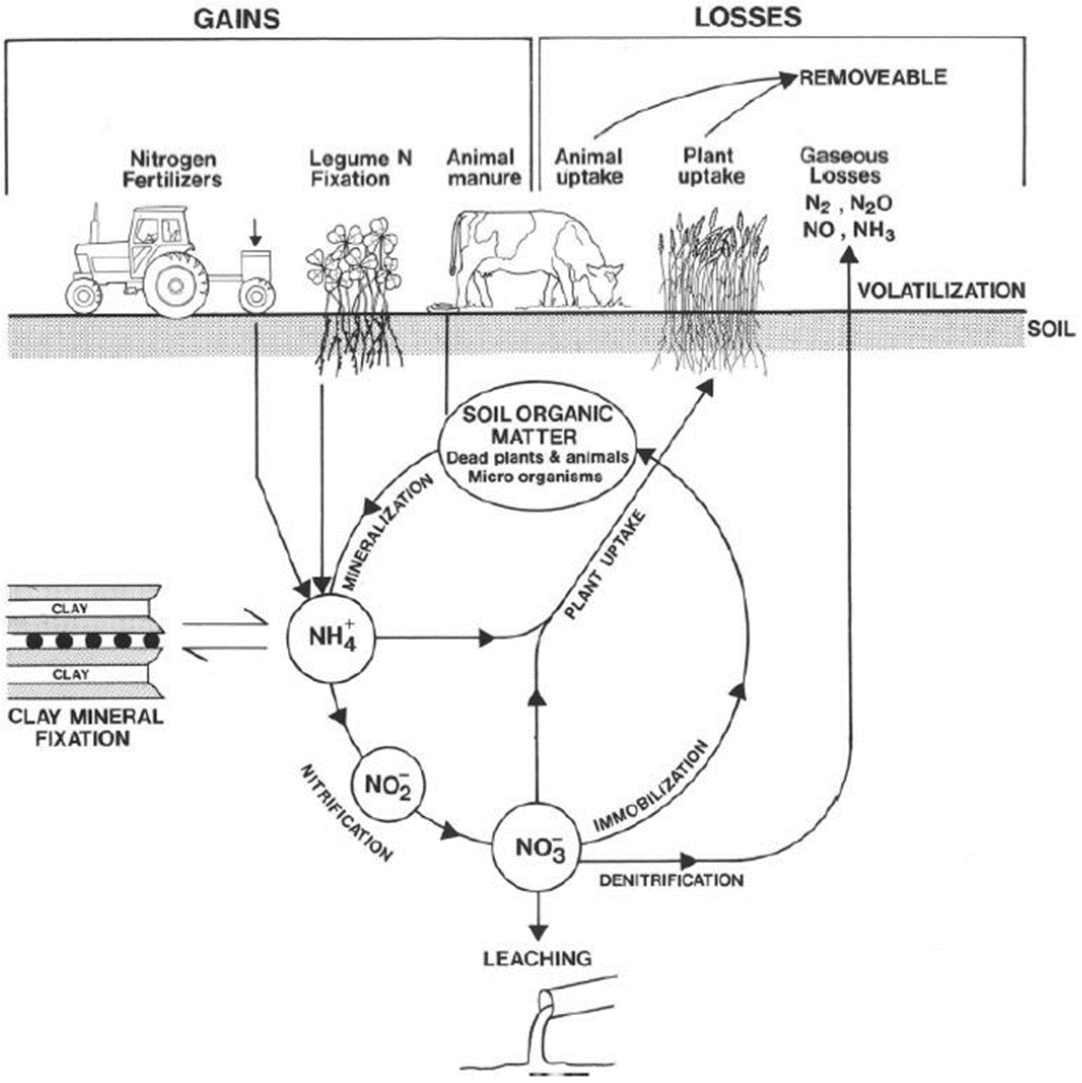


Figure 2.1 A generalized diagram of the nitrogen cycle in agricultural soils from Di and Cameron (2002a)

2.1.2.1 Nitrogen Inputs as Urine

After consumption of high N content herbage during grazing, a small fraction of the N consumed is retained and used by ruminant livestock, and the rest is excreted as dung or urine directly on to the pasture (Whitehead 1995). Dairy cattle may urinate up to 12 times a day, averaging 2 L per urination event (Selbie et al. 2015a). Each urine patch covers an average area of 0.2-0.5 m^2 (Whitehead 1995) but the area around the urine patches are also affected. Estimates of the affected average area of a urine patch range from 0.68 m^2 (Selbie et al. 2015a) to 0.95 m^2 (Buckthought et al. 2016), or up to 3.4 times larger than the wetted area (Buckthought et al. 2016). Urine patches can cover an area equivalent to ~20% of the pasture annually (Moir et al. 2011).

Cattle urine is high in urea, which accounts for ~60-90% of the total N in urine (Doak 1952, Haynes and Williams 1992, Whitehead 1995). In much smaller concentrations, urine also includes a number of other nitrogenous compounds including hippuric acid, allantoin, uric acid, xanthine, hypoxanthine, creatin and creatinine (Whitehead 1995). The N loading under a urine patch can reach up to 1000 kg N ha^{-1} after a single urine deposition event (Haynes and Williams 1993), depending on the diet and water intake of the cow (Betteridge and Andrews 1986). This high N loading rate under a urine patch is far in excess of grass plant requirements, which are 300-700 kg N ha^{-1} annually (Moir et al. 2007). As a consequence, there is a pool of excess N available for microbial uptake and transformation. Microbial and chemical transformations of urine N change the form of N and can alter the soil environment (Figure 2.1).

Following urine deposition, hydrolysis (Figure 2.2) converts the urea in the urine to ammonium (NH_4^+) within ~ 48 hours (Doak 1952, Haynes and Williams 1992), which can raise the soil pH by 3 units (Jarvis and Pain 1990, van Groenigen et al. 2005a). The NH_4^+ is then available for plant uptake, immobilization, or nitrification. During nitrification, microbes convert NH_4^+ to NO_3^-. Nitrification releases H^+ ions thereby lowering soil pH (Sherlock and Goh 1984). Ammonia (NH_3) production after urine deposition means that there is also potential for nitrite (NO_2^-) accumulation in the soil during nitrification (Van Cleemput and Samater 1995, Venterea et al. 2015). The NO_3^- created from nitrification may be taken up by plants, leached, or reduced to gaseous forms such as nitric oxide (NO), N_2O or N_2 via denitrification (Ball et al. 1979, Whitehead and Bristow 1990).

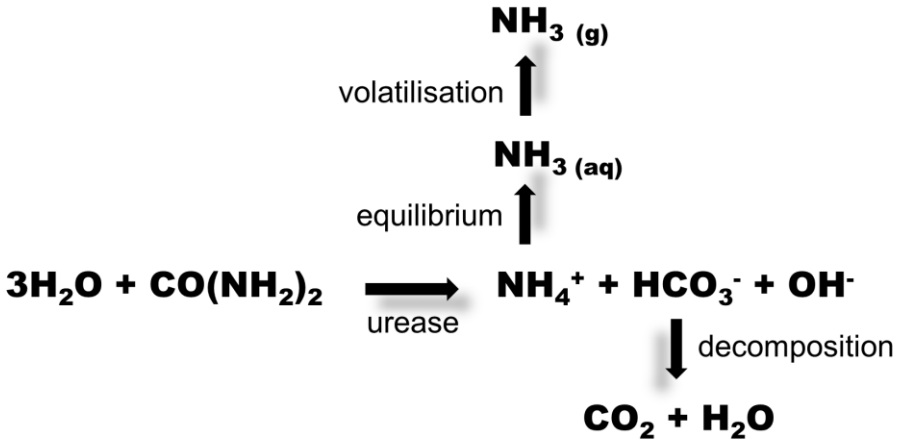


Figure 2.2 The chemical equation for urea hydrolysis which happens after urine deposition, adapted from Sherlock et al. (2011) and Sherlock (1984)

2.1.2.2 Environmental Conditions in Grazed Pastures

Environmental conditions largely determine plant growth and microbial processes in the soil. These then determine the cycling and fate of N in pasture and soils. Upwards of 95% of the N in New Zealand pasture soils is organic material from decomposing plant material and microbial biomass, and is thus not available for plant uptake (Haynes 1986). The inorganic, plant available N (NO_3^-, NH_4^+, and NO_2^-), only accounts for < 2% of the total soil N (Haynes 1986). While grazing helps change the composition of N forms and distribution of N in the soil due to the role of excretal returns, grazing can also affect soil physical and chemical properties in a number of ways, beyond simply changing N substrate supply.

Urine deposition may decrease soil O_2 concentrations. The water embodied in the urine represents an addition of water to soil that can replace air-filled pores with water, reducing O_2 availability. The hydrolysis reactions that occur after urine deposition may also decrease soil O_2 (Figure 2.2). Following urine deposition, pulses of CO_2 have been observed (Uchida et al. 2008, Ma et al. 2015) and these have been attributed to carbonate hydrolysis or respiration (Sherlock 1984, Chadwick and Pain 1997, Saggar et al. 2004). This may cause a decrease in soil O_2 concentrations and influence N cycling pathways. Decreased soil O_2 concentrations may induce anaerobic denitrification processes and may explain the pulse of N_2O emissions sometimes observed within 24-48 hours after urine deposition (van Groenigen et al. 2005a, Uchida et al. 2008, Orwin et al. 2010). However, the extent and duration of the effects of urine deposition on soil O_2 are unknown because no studies have measured soil O_2 under a urine patch, despite the implications for N_2O emissions and N cycling.

Few measurements of soil O_2 concentrations in pastures soils exist. Previous studies that have measured soil O_2 concentrations in pasture soils noted low variability in O_2 concentrations in non-saturated soil conditions (Simojoki and Jaakkola 2000). After saturating soils, it can take days for O_2 concentrations in the bulk soil to drop below 10%, with this time dependent on soil texture (Eccles et al. 1990). These previous pasture measurements were sporadic. Instead of direct soil O_2 measurements, soil moisture is often used as a proxy for soil O_2, with water-filled pore space (WFPS) commonly used (Linn and Doran 1984). Farquharson and Baldock (2008) have noted the use of total porosity in the calculation of WFPS makes it a poor descriptor for soils with different bulk densities as it does not represent the fraction of the entire soil volume available for air or water, leading to different WFPS and air-filled pore space (AFPS) in soil's with different bulk

densities but with the same water content. This distorts the relationships between WFPS and N_2O fluxes when soil compaction varies (Balaine et al. 2013).

Pastures are generally considered to be aerobic, but cattle treading can compact the soil, leading to a decrease in soil aeration (Menneer et al. 2005). The degree of compaction increases with higher stocking rates and with wetter soil conditions when the soil is more malleable (Cournane et al. 2011). Post-grazing, the increases in compaction and decreased aeration have been found to last ~3 days in a silty loam with impeded subsoil drainage (Menneer et al. 2005). This has implications for the processes involved in soil GHG exchange with the atmosphere.

Compaction reduces the total porosity and increases tortuosity (McDowell et al. 2003, Houlbrooke et al. 2008, Cournane et al. 2011). It impedes the transfer of gases between the soil and the atmosphere because it will be more difficult for O_2 in the soil to be replenished by the atmosphere following respiration, and for gases produced in the soil to be emitted into the atmosphere, until soil conditions change. Soil gas diffusion is affected by soil moisture content as gas diffusion is 10^{-4} times slower in water than in air (Farquharson and Baldock 2008) so it will be affected by precipitation and land management practices such as irrigation. The effect of rainfall on gas diffusion in soils, for example, depends on the rainfall rate and duration, as well as the soil's pore size distribution, which in turn is affected by the soil bulk density. These factors determine the water storage capacity and drainage rate of the soil (Cournane et al. 2011).

Improved understanding of environmental conditions that control the timing and magnitude of N_2O emissions from agricultural soils are required. This information may help inform land management strategies to reduce N_2O emissions. Below is a review of the potential pathways for N cycling, and N_2O production, N_2 production, or N_2O uptake due to N_2O reductase (N_2OR) activity in soils, and the environmental factors that control them.

2.2 Pathways for N_2O Production and Uptake (N_2OR)

Abiotic mechanisms for N_2O production are described by **chemodenitrification**. Biological mechanisms for N_2O production are attributed to **co-denitrification**, **nitrification**, **nitrifier-denitrification**, **denitrification**, or a **coupling of these processes**. Other biological processes, such as **dissimilatory nitrate reduction to ammonium**, are also mentioned below, but the prior processes are given more attention because they are more likely to be responsible for N_2O and N_2 emissions in pasture soils (Figure 2.3).

Chemodenitrification: At low pH, abiotic production of NO, N_2O, or N_2 production can occur from chemodenitrification. Nitrite is protonated to form nitrous acid (HNO_2) at low pH (Chalk and Smith 1983, Van Cleemput and Samater 1995, Bremner 1997, Donaldson et al. 2014). When the soil NO_2^- : HNO_2 equilibrium shifts in favor of HNO_2, HNO_2 either self decomposes to form NO, nitric acid (HNO_3), and water (H_2O), or reacts with soil organic matter, hydroxylamine (NH_2OH), or readily available cations to form NO, N_2, N_2O and CH_3ONO (Chalk and Smith 1983, Van Cleemput and Samater 1995, Bremner 1997, van Cleemput 1998, Venterea and Rolston 2000, Zhu et al. 2013).

Co-dentrification: Co-dentrification is a hybrid process carried out by bacteria, including autotrophic nitrifiers (Firestone and Davidson 1989, Wrage et al. 2001, Long et al. 2013), and fungi (Long et al. 2013). During this process, N_2O or N_2 are produced through the reduction of NO_2^- and organic N compounds such as azide, salicylhydroxamine acid, and NH_2OH (Firestone and Davidson 1989, Wrage et al. 2001, Spott et al. 2011). During this process, both the NO_2^- and the organic N compound each contribute one atom of N to N_2O (Müller et al. 2014). Little is known about this process, but it has recently been reported to make a significant contribution to N_2 emissions from Irish pasture soils (Selbie et al. 2015b).

Nitrification: Nitrification has long been considered a two-step process whereby ammonia oxidizers, (step 1) either bacteria (AOB), or archaea (AOA), or anammox organisms, convert NH_4^+ or NH_3 to NO_2^-, (step 2) then nitrite oxidizing bacteria (NOB) convert this NO_2^- to NO_3^- (Sahrawat 2008, Ward 2013). The AOB produce N_2O as a minor intermediate product during oxidation of NH_3 to NO_2^- from NH_2OH (Ward 2013). The two steps of nitrification can run at different rates. While both AOB and NOB are sensitive to NH_3, lags in NOB activity can occur resulting in NO_2^- accumulation in soil (Anthonisen et al. 1976, Van Cleemput and Samater 1995, Venterea et al. 2015). This may happen following urine deposition because of impaired NO_2^- oxidation due to the urea hydrolysis, which creates NH_3 (Zhu et al. 2013). Recently, microbes that can complete both steps of nitrification, called completed ammonia oxidizers, or comammox bacteria, belonging to the genus *Nitrospira*, have been found in biofilms (Santoro 2016).

Nitrifer-denitrification: Nitrifer-denitrification is the reduction of NH_4^+ to NO, N_2O, or N_2 (Poth and Focht 1985, Wrage et al. 2001) by a group of autotrophic AOB who complete all steps of the process (Wrage et al. 2001). Nitrifier-denitrification rates increase with low O_2 (Whittaker et al. 2000, Dundee and Hopkins 2001, Kool et al. 2011a, Zhu et al. 2013).

Denitrification: Denitrification is the sequential biological reduction of NO_3^- to NO_2^-, NO, N_2O, and N_2 and is performed by facultative aerobes which switch from O_2 respiration to denitrification under O_2 limited conditions (Zhu et al. 2013), and when there is an N oxide available to be used in place of O_2 as a terminal electron acceptor (Zumft 1997). Not all denitrifying microbes have the cellular machinery to complete all steps of denitrification (Sanford et al. 2012), and a broad range of microbes, including fungi, prokaryotes, bacteria, and archaea, have the ability to partially denitrify (Zumft 1997, Sanford et al. 2012).

Dissimilatory nitrate reduction to ammonium: Dissimilatory NO_3^- reduction to NH_4^+ (DNRA) is completed by facultative and obligatory fermentative bacteria (Tiedje 1988, Silver et al. 2001) and can produce N_2O as a by-product under reducing conditions. This process is typically found in anaerobic sludge, lake littoral sediments, and riparian wetlands. Like denitrification, DNRA occurs under O_2 limited conditions, but conditions need to be more reducing for DNRA compared to denitrification (Matheson et al. 2002).

Coupling of biological processes: The above biological processes occur simultaneously in the soil. Simultaneous nitrification and denitrification, for example, can contribute to N_2O emissions from soils (Wrage et al. 2001, Pihlatie et al. 2004), especially in soil microsites, or at aerobic-anaerobic interfaces (Baldwin and Mitchell 2000, Wrage et al. 2001). During these instances, denitrifiers can use NO_2^- or NO_3^- produced during nitrification under aerobic conditions in proximate areas of the soil (Parkin 1987, Smith et al. 1998). Coupling of processes resulted in a high degree of spatial and temporal variability of N_2O emissions (Kuenen and Robertson 1994, Hergoualc'h et al. 2007).

Other pathways: There are a number of other identified biological pathways for N_2O and N_2 production that little is known about. During nitrification, autotrophic anammox bacteria can oxidize NH_4^+ using NO_2^- instead of O_2 under anaerobic conditions (Ward 2013). They do not produce N_2O, but can produce N_2 instead of NO_2^- (Kuenen 2008), representing a loss of fixed N equivalent to complete denitrification. Ammonia oxidizing archaea can produce N_2O (Ward 2013), and strains of *nitrobacter* can produce N_2O via anaerobic reduction of NO_3^- (Freitag et al. 1987, Wrage et al. 2001, Ward 2013).

Each step of these biological N transformation processes is regulated by a different enzyme. These are synthesized by different groups of functional genes (Table 2.1, Figure 3.3). The regulation of these genes is influenced by environmental conditions.

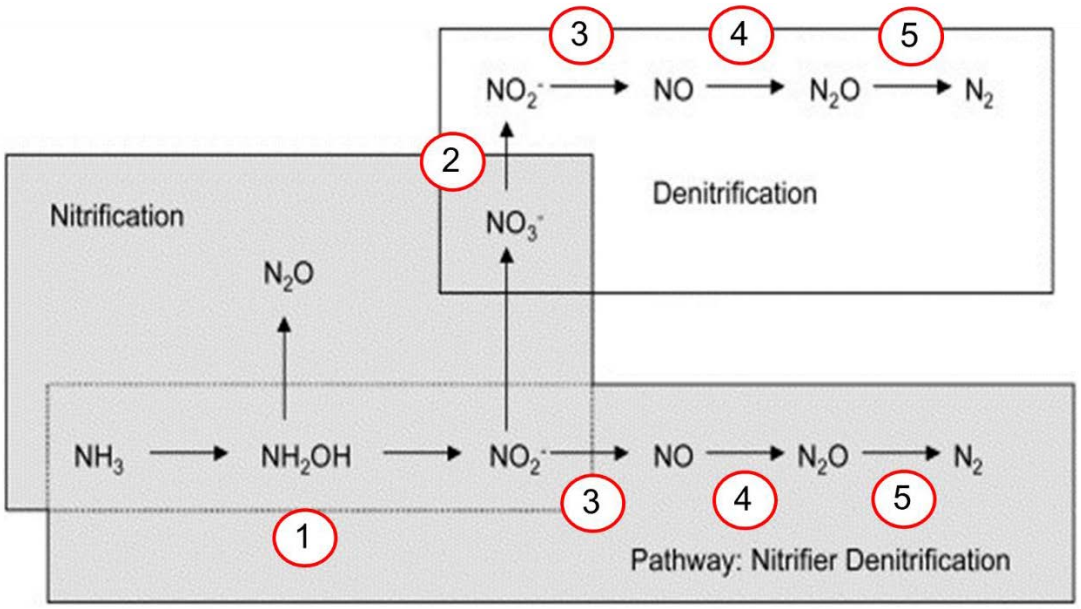


Figure 2.3 Microbial N transformations producing N_2O emissions (Wrage et al., 2001). The numbers refer to the reactions listed in Table 2.1.

Table 2.1 Enzymes, functional genes, and redox potential for steps in nitrification, nitrifier denitrification, and denitrification

step	reaction	enzymes	functional genes	redox potential (E'_0)
(1)	$NH_3/NH_4^+ \rightarrow NO_2^-$	ammonia monooxygenase	amoA [2]	
(2)	$NO_3^- \rightarrow NO_2^-$	membrane-bound nitrate reductase [1]	narG [1]	+ 420 mV [3]
		periplasmic nitrate reductase [1]	napA [1]	
(3)	$NO_2^- \rightarrow NO$	Cd_1 nitrite reductase [1]	nirS [1]	+ 375 mV [3]
		Cu nitrite reductase [1]	nirK [1]	
(4)	$NO \rightarrow N_2O$	nitric oxide reductase [1]	norB [1]	+ 1175 mV [3]
		Quinol nitric oxide reductase [1]	qnorB [1]	
(5)	$N_2O \rightarrow N_2$	nitrous oxide reductase [1]	nosZ [1]	+ 1355 mV [3]

[1] Zumft (1997), [2] Ward (2013), [3] Ferguson and Richardson (2004)

2.2.1 Environmental Regulators of N_2O and N_2 Production

It is out of the scope of this thesis to discuss how environmental factors influence each N_2O production pathway in detail. Land use may also influence the soil microbial community composition (Fierer et al. 2003, Cookson et al. 2007, Ma et al. 2015) but these shifts in microbial communities with land use change and soil type are not covered here.

The effects of environmental variables on the functioning and rates of microbial processes, and the gaseous end-products, confounds the interpretation of monitoring campaigns under uncontrolled conditions. For example, an increase in N_2O emissions may not relate to an increase in denitrification in soil. It may instead be the result of a decrease in N_2OR activity resulting in a greater proportion of N being emitted during denitrification as N_2O.

The influence of environmental variables have on nitrification, nitrifer-denitrification, and denitrification, and N_2O and N_2 production from these processes, are summarized in Table 2.2. Literature suggests denitrification, nitrifier denitrification, and sometimes nitrification, are responsible for a majority of N_2O production in soil (Butterbach-Bahl et al. 2013b, Signor et al. 2013), and increases in N_2O production from these processes increase under low O_2 concentrations (see Table 2.2 for references) and are also affected by other environmental factors.

Table 2.2 The relationships between biological soil processes that can produce N_2O and N_2, and environmental variables. The direction of the relationship between either increases (indicated by ↑) or decreases (indicated by ↓) in the environmental variables, and increases (indicated by ↑) in the microbial processes, and N_2O and N_2 production.

variable	↑Nitrification	↑Nitrifier-Denitrification	↑Denitrification	↑N_2O	↑N_2
C		↓[8]	↑[3,4]	↑[4]	↓[4]
NO_3^-			↑[3,4]	↑[3]	↓[3]
NH_4^+	↑[3]				
NO_2^-		↑[10,13]		↑[14]	
pH	↑[15]	↓[8]	↑[2]	↑[2,19]	↑[2,19,20]
soil moisture	↓[6]	↑[9]	↑[21,22]	↑[21,22]	↑[23]
O_2	↑[6,16]	↓[8,10,11,12,13]	↓[3,4]	↓[17,18]	↓[3,4,8]
temperature	↑[1]	↑	↑[1]	↑[7]	↓[7]

[1] Barnard et al. (2005), [2] Šimek and Cooper (2002), [3] Knowles (1982), [4] Morley and Baggs (2010), [5] Zhu et al. (2013), [6] Ward (2013), [7] Owens et al. (2015), [8] Wrage et al. (2001), [9] Kool et al. (2011b), [10] Poth and Focht (1985), [11] Whittaker et al. (2000), [12] Dundee and Hopkins (2001), [13] Kester et al. (1997), [14] Wrage et al. (2004), [15] Sahrawat (2008), [16] Khalil et al. (2004), [17] Davidson and Schimel (1995), [18] Goreau et al. (1980), [19] Bakken et al. (2012), [20] Bergaust et al. (2012), [21] Linn and Doran (1984), [22] Dobbie et al. (1999), [23] Ruser et al. (2006)

Nitrification both alters, and is controlled by, the soil environment. Nitrification is inhibited at low pH. Soil pH decreases (acidification) during nitrification due to the release of H^+ ions (Sahrawat 2008). Gross rates of nitrification slow down with decreased O_2 concentrations (Khalil et al. 2004). However, N_2O production from nitrification may increase under these low O_2 conditions (Goreau et al. 1980, Davidson and Schimel 1995). Nitrous oxide from nitrification is "an exception rather

than a rule" (Stevens and Laughlin 1998) and other pathways are likely to contribute a greater proportion of the total N_2O emission (Zhu et al. 2013).

In general, denitrification requires a labile form of C as an energy source, and NO_3^- as an electron acceptor (Šimek and Cooper 2002). Nitrifier-denitrification also needs an electron acceptor, such as NO_2^- (Wrage et al. 2004) or NH_4^+ (Zhu et al. 2013). The relative proportion of N_2O emitted from denitrification compared to nitrifier-denitrification, as well as the amount of N_2O, increases as soil O_2 decreases (Goreau et al. 1980, Venterea 2007, Zhu et al. 2013).

While pH is sometimes called the "master variable" determining microbial functioning (Šimek and Cooper 2002, Qu et al. 2014) because of its influence on both the rates of microbial processes and the gaseous end-products produced (Šimek and Cooper 2002, Sahrawat 2008, Bakken et al. 2012), O_2 concentration may be the master variable for enzyme synthesis and activity. Oxygen has long been known to be a proximal controller of N_2O and N_2OR (Firestone et al. 1980, Knowles 1982, Firestone and Davidson 1989). Soil O_2 can be affected by grazing induced-compaction, which can change the ratio of N_2O:N_2 and thus net N_2O soil emissions (Ruser et al. 2006, Thomas et al. 2008, Harrison-Kirk et al. 2015). The redox requirements for each step of denitrification (Table 2.1) are not so low that the process cannot happen in anaerobic microsites in soils. Low redox is important for denitrification, otherwise O_2 will out compete NO_3^- as a terminal electron acceptor (Firestone and Davidson 1989). However, few studies have actually measured soil O_2 along with N_2OR, despite the linkage between O_2 and N_2OR enzyme regulation.

2.3 Nitrous Oxide Reductase

Nitrous oxide reductase activity depends on how environmental factors influence N_2OR, and the relative abundance of microbes with N_2OR which in turn dictates the ratio of N_2O:N_2 produced. This influences the net N_2O fluxes from soils. Thus, the expression of the nosZ gene, which encodes for N_2OR is of particular interest.

Much of what is known about N_2OR has been learned from the bacterial species *Paracoccus denitrifican* which is commonly used as a model organism for understanding the microbial aspects of denitrification (Bergaust et al. 2012). The reduction of N_2O to N_2 during denitrification requires two protons and two electrons (Pauleta et al. 2013):

$$N_2O + 2H^+ + 2e^{-1} \rightarrow N_2 + H_2O,\ E^o\ (pH\ 7.0) = 1.32V,\ \Delta G^{0\prime} = -339.5\ kJ\ mol^{-1} \qquad \text{Equation 2.1}$$

The ATP (energy yield) and growth yield from O_2 respiration is significantly higher than that of denitrification for microbes. This means that it is energetically more efficient to down-regulate, or stop synthesizing, the denitrification enzymes in the presence of O_2 (Strohm et al. 2007). Denitrification lets bacteria survive under anaerobic conditions (Fazzolari et al. 1998).

Nitrous oxide reductase (N_2OR) is the only known enzyme capable of reducing N_2O into N_2 (Thomson et al. 2012) and thus is the only known pathway for terrestrial uptake of N_2O. In soil, denitrifiers make up only 5-6% of the total proportion of bacteria (Henry et al. 2006). However, N_2OR is not universally carried by all denitrifiers, with studies reporting 37% of bacterial denitrifiers lacking nosZ genes for expressing N_2OR (Jones et al. 2008). A variety of denitrifying and non-denitrifying microbes from sixteen taxonomic groups of bacteria and archaea, from a variety of environments, have been found to have functional nosZ gene encoding (Sanford et al. 2012).

Nitrous oxide reductase is a soluble dimeric enzyme (Saunders et al. 2000). As shown in Figure 2.4, the genes for N_2OR are located in the periplasm of the cell (Pauleta et al. 2013). There are two types of N_2OR. The first is the simple z-type, which is encoded by the nosZ gene. The other type is a "3 domain" z-type N_2OR sequence which has been detected in only a few organisms (Pauleta et al. 2013).

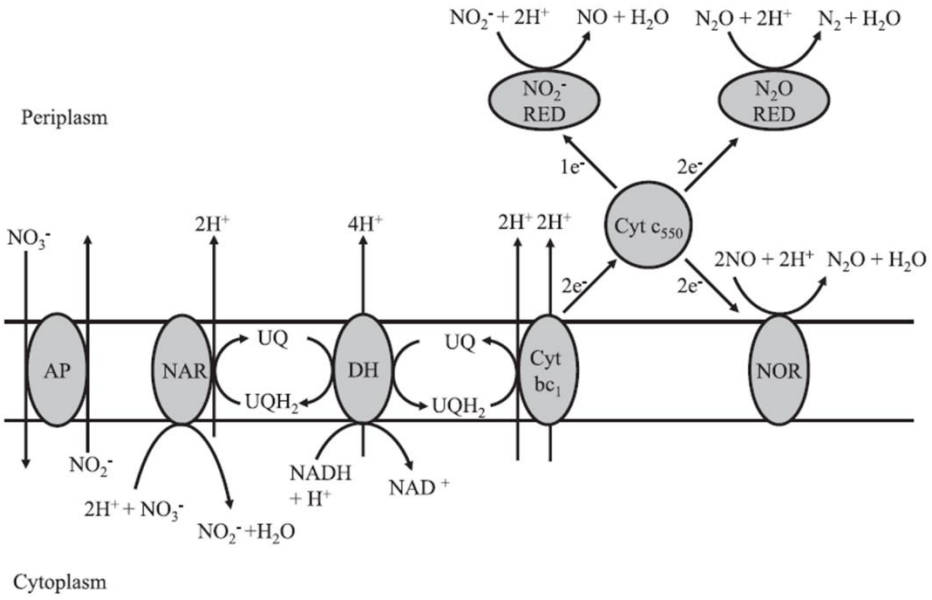


Figure 2.4 Organization of respiratory elements involved in denitrification by Paracoccus denitrificans. The figure is from (Strohm et al. 2007). AP, antiporter; NAR, nitrate reductase b; UQ, ubiquinone; DH, NADH dehydrogenase; Cyt bc1, cytochrome bc1 complex; NOR, NO reductase; NO_2 RED, nitrite reductase; N_2O RED, N_2O reductase; Cyt c550, cytochrome c550.

The N_2OR is inhibited by low pH and the presence of O_2 (Knowles 1982). Nitrous oxide reductase is more sensitive to the presence of O_2 than the other enzymes involved in denitrification (Knowles 1982, Wrage et al. 2001). It is held that N_2O is reduced to N_2 only under low O_2 conditions (Morley et al. 2008, Russow et al. 2009, Morley and Baggs 2010). However, Wu et al. (2013) found that N_2O was still reduced to N_2 when dry soil was incubated with 21% O_2. The presence of O_2 may not stop the reactions of the enzymes that have already been produced (Zumft 1997). These results are in contrast to other findings which have reported that both transcriptional and post-transcriptional enzyme synthesis stops at high O_2 concentrations (Bakken et al. 2012).

2.3.1 Measuring Nitrous Oxide Reductase

Measurement of N_2 along with N_2O would help inform activity of N_2OR in soil. However, N_2 fluxes from soil are difficult to measure due to high ambient N_2 concentrations. Quantification of N_2OR activity is important because it provides insight into the ratio of N_2O:N_2 produced during denitrification. Alternatives to direct measurements of N_2 are often used to understand how N_2OR dynamics influences net N_2O emissions. Molecular techniques for detecting N_2OR involve measuring gene abundance and gene expression of nosZ genes using techniques such as quantitative polymerase chain reactions (Deslippe et al. 2014), however, these methods are not covered in this review. There are non-molecular methods for detecting N_2OR, including the use of acetylene (C_2H_2) inhibition and ^{15}N isotopes which are briefly reviewed below.

2.3.1.1 Acetylene

Acetylene (C_2H_2) inhibits the reduction of N_2O to N_2 at 10 kPa, making N_2O the terminal end-product of denitrification (Yoshinari and Knowles 1976). The limitations of C_2H_2 are well documented. It has been shown to lead to an underestimation of denitrification rates by scavenging NO, an obligate intermediate to N_2O production, and restricting NO_3^- production by inhibiting nitrification (Bollmann and Conrad 1997, Groffman et al. 2006). It has also been found to enhance C respiration, and some microbes have the ability to metabolize C_2H_2 making it an ineffective inhibitor for microbes with that capability (Groffman et al. 1999, Groffman et al. 2006). Some studies have employed C_2H_2 as a relatively inexpensive way to assess the basal denitrification rates, or how much N_2O is being reduced to N_2 in indigenous soil conditions (Ryden and Dawson 1982, Stevenson et al. 2011). By only using soil homogenized samples rather than intact samples diffusion of the substrates and C_2H_2 into the soil can be maximized (Drury et al. 2008). This test uses C_2H_2 in the same way as it is used in a denitrification enzyme assay (DEA),

however, during DEA's, additional substrates are added to create "un-limiting" conditions (Drury et al. 2008). Specifically, DEA's are preformed adding C and NO_3^-, and incubating soils under anaerobic conditions; conditions which may be rarely present in soil (Groffman et al. 1999). Because of this, the denitrification potential derived from DEA's is not a representation of the actual denitrification rates in the soils, but they can still provide insight into microbial functioning in soil. The benefit of C_2H_2 inhibition for determining denitrification potential using DEA's is that it is an inexpensive and accessible method that can isolate N_2OR dynamics, independent of the lack of optimum nutrient or O_2 conditions. Despite its shortcomings, the use of C_2H_2 to determine basal denitrification rates and denitrification potential in soil has proved to be a useful indexing tool for comparing denitrification activity between soils and under different soil conditions (Groffman et al. 1999, Burgin and Groffman 2012).

2.3.1.2 Nitrogen Stable Isotopes

The stable N isotope can be used to measure N_2O and N_2 by adding labelled ^{15}N labelled substrate to soil. The premise of this method is that in nature, N has two stable isotopes; ^{14}N, which makes up approximately 99.6337% of natural N, and ^{15}N, which has an extra neutron compared to ^{14}N, and makes up approximately 0.3663% of N (Schoeller 1999, Dawson and Brooks 2001). The heavier stable isotope ^{15}N is discriminated against in favour of ^{14}N, during biochemical, biogeochemical and physiological processes, due to its greater atomic mass (He et al. 2009). The primary advantage of ^{15}N is that the addition of ^{15}N enriched N sources can be used to monitor the proportion of N_2O and N_2 emitted from the added, ^{15}N pool (Stevens and Laughlin 1998, Müller et al. 2002). Sample analysis is expensive, and field conditions introduce variability into the measurements (Mathieu et al. 2006), so this method is best confined to controlled studies.

2.4 Scope and Outline

This chapter started with an overview of dairying farming and irrigation. A summary of the N cycling in grazed pastures and the processes responsible for N_2O production and N_2OR synthesis, and the non-molecular methods used to infer or measure N_2OR, were presented.

Various environmental factors including inorganic C and O_2 work in concert to influence the rates of microbial processes and the ratio of the gaseous end products, as determined by N_2OR activity. Few studies have measured pasture soil O_2 concentrations, and there are no measurements of soil O_2 from under a urine patch, despite the linkage between N_2OR and O_2. Soil moisture is often used

as a proxy for soil O_2. There are some concerns about the validity of this substitution because commonly used soil moisture measures, such as WFPS, do not account for differences in soil properties. There is the potential for denitrification in anaerobic microsites in otherwise aerobic soils, suggesting the potential for N_2OR in lightly irrigated grazed pastures, however, no studies have measured soil O_2 and N_2OR along with N_2O in an irrigated grazed pasture. This is the focus of Experiment 1, Chapter 4.

2.5 References

Anthonisen, A. C., R. C. Loehr, T. B. S. Prakasam, and E. G. Srinath. 1976. Inhibition of Nitrification by Ammonia and Nitrous Acid. Journal Water Pollution Control Federation 48:835-852.

Bakken, L., L. Bergaust, B. Liu, and A. Frostegård. 2012. Regulation of denitrification at the cellular level: a clue to the understanding of N_2O emissions from soils. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences 367:1226-1234.

Balaine, N., T. Clough, M. Beare, S. Thomas, E. Meenken, and J. Ross. 2013. Changes in Relative Gas Diffusivity Explain Soil Nitrous Oxide Flux Dynamics. Soil Science Society of America Journal 77:1496-1505.

Baldwin, D. S., and A. M. Mitchell. 2000. The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river–floodplain systems: A synthesis. Regulated Rivers: Research & Management 16:457-467.

Ball, R., P. W. Keeney, P. W. Theobald, and P. Nes. 1979. Nitrogen Balance in Urine-affected Areas of a New Zealand Pasture. Agronomy Journal 71:309-314.

Barnard, R., P. Leadley, and B. Hungate. 2005. Global change, nitrification, and denitrification: A review. Global Biogeochemical Cycles 19:GB1007.

Bergaust, L., R. van Spanning, A. Frostegård, and L. Bakken. 2012. Expression of nitrous oxide reductase in Paracoccus denitrificans is regulated by oxygen and nitric oxide through FnrP and NNR. Microbiology 158:826-834.

Betteridge, K., and W. G. K. Andrews. 1986. Intake and excretion of nitrogen, potassium and phosporus by grazing steers. Journal of Agricultural Science 106:393-404.

Bollmann, A., and R. Conrad. 1997. Acetylene blockage technique leads to underestimation of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide. Soil Biology and Biochemistry 29:1067-1077.

Bouwman, A. F., L. J. M. Boumans, and N. H. Batjes. 2002. Emissions of N_2O and NO from fertilized fields: Summary of available measurement data. Global Biogeochemical Cycles 16:1058.

Bremner, J. 1997. Sources of nitrous oxide in soils. Nutrient Cycling in Agroecosystems 49:7-16.

Buckthought, L. E., T. J. Clough, K. C. Cameron, H. J. Di, and M. A. Shepherd. 2016. Plant N uptake in the periphery of a bovine urine patch: determining the 'effective area'. New Zealand Journal of Agricultural Research:1-19.

Burgin, A., and P. Groffman. 2012. Soil O2 controls denitrification rates and N_2O yield in a riparian wetland. Journal of Geophysical Research 117:G01010.

Butterbach-Bahl, K., E. Baggs, M. Dannenmann, R. Kiese, and S. Zechmeister-Boltenstern. 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philosophical Transactions of the Royal Society of London B: Biological Sciences 368:20130122.

Carran, R. A., and T. Clough. 1996. Environmental impacts of nitrogen in pastoral agriculture. Agronomy Society of New Zealand Special Publication 11:99-102.

Chadwick, D. R., and B. F. Pain. 1997. Methane fluxes following slurry applications to grassland soils: laboratory experiments. Agriculture, Ecosystems & Environment 63:51-60.

Chalk, P. M., and C. J. Smith. 1983. Chemodenitrification. Pages 65-89 in J. R. Freney and J. R. Simpson, editors. Gaseous Loss of Nitrogen from Plant-Soil Systems. Springer Netherlands.

Clough, T. J., F. M. Kelliher, R. R. Sherlock, and C. D. Ford. 2004. Lime and Soil Moisture Effects on Nitrous Oxide Emissions from a Urine Patch. Soil Science Society of America Journal 68:1600-1609.

Cookson, W. R., M. Osman, P. Marschner, D. A. Abaye, I. Clark, D. V. Murphy, E. A. Stockdale, and C. A. Watson. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biology and Biochemistry 39:744-756.

Cournane, F., R. McDowell, R. Littlejohn, and L. Condron. 2011. Effects of cattle, sheep and deer grazing on soil physical quality and losses of phosphorus and suspended sediment losses in surface runoff. Agriculture, Ecosystems and Environment 140:264-272.

Davidson, E. A., and J. P. Schimel. 1995. Microbial processes of production and consumption of nitric oxide, nitrous oxide, and methane. Pages 327-357 in Methods in Ecology: Trace Gases. Blackwell Scientific.

Dawson, T., and P. Brooks. 2001. Fundamentals of Stable Isotope Chemistry and Measurement. Pages 1-18 in M. Unkovich, J. Pate, A. McNeill, and Gibbs, editors. Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems. Springer Netherlands.

de Klein, C., and S. Ledgard. 2005. Nitrous Oxide Emissions from New Zealand Agriculture – Key Sources and Mitigation Strategies. Nutrient Cycling in Agroecosystems 72:77-85.

Deslippe, J., H. Jamali, N. Jha, and S. Saggar. 2014. Denitrifier community size, structure and activity along a gradient of pasture to riparian soils. Soil Biology and Biochemistry 71:48-60.

Di, H. J., and K. C. Cameron. 2002. Nitrate leaching in temperate agroecosystems: Sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64:237-256.

Doak, B. W. 1952. Some chemical changes in the nitrogenous constituents of urine when voided on pasture. The Journal of Agricultural Science 42:162-171.

Dobbie, K. E., I. P. McTaggart, and K. A. Smith. 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research 104:26891-26899.

Dodd, M. B., J. R. Crush, A. D. Mackay, and D. J. Barker. 2011. The "root" to more soil carbon under pastures. Proceedings of the New Zealand Grassland Association 73:43-50.

Donaldson, M., D. Bish, and J. Raff. 2014. Soil surface acidity plays a determining role in the atmospheric-terrestrial exchange of nitrous acid. Proceedings of the National Academy of Sciences 111:18472-18477.

Drury, C. F., D. D. Myrold, E. G. Beauchamp, and W. D. Reynolds. 2008. Denitrificaiton techniques for soil. Pages 471-493 in M. R. Carter and E. G. Gregorich, editors. Soil Sampling and Methods of Analysis. CRC Press, Boca Raton, FL, USA.

Dundee, L., and D. W. Hopkins. 2001. Different sensitivities to oxygen of nitrous oxide production by Nitrosomonas europaea and Nitrosolobus multiformis. Soil Biology and Biochemistry 33:1563-1565.

Eccles, W. J., C. Matthew, and A. C. P. Chu. 1990. Response of Matua prairie grass and Ellett perennial ryegrass to excess soil moisture in sand, silt and clay soils. Proceedings of the New Zealand Grassland Association 51:127-130.

Farquharson, R., and J. Baldock. 2008. Concepts in modelling N_2O emissions from land use. Plant and Soil 309:147-167.

Fazzolari, É., B. Nicolardot, and J. Germon. 1998. Simultaneous effects of increasing levels of glucose and oxygen partial pressures on denitrification and dissimilatory nitrate reduction to ammonium in repacked soil cores. European Journal of Soil Biology 34:47-52.

Ferguson, S. J., and D. J. Richardson. 2004. Electron transport system for reduction of nitrogen oxides and nitrogen oxyanions. Pages 169-206 in D. Zannoni, editor. Respiration in Archaea and Bacteria: Diversity of Prokaryotic Respiratory Systems. Kluwer Academic Publisher, The Netherlands.

Fierer, N., J. P. Schimel, and P. A. Holden. 2003. Influence of Drying–Rewetting Frequency on Soil Bacterial Community Structure. Microbial Ecology 45:63-71.

Firestone, M., and E. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil.in M. O. Andreae and D. S. Schimel, editors. Exchange of Trace Gases Between Ecosystems and the Atmosphere. Wiley, New York.

Firestone, M. K., R. B. Firestone, and J. M. Tiedje. 1980. Nitrous oxide from soil denitrification: Factors controlling its biological production. Science 208:749-751.

Freitag, A., M. Rudert, and E. Bock. 1987. Growth of Nitrobacter by dissimilatoric nitrate reduction. FEMS Microbiology Letters 48:105-109.

Goreau, T. J., W. A. Kaplan, S. C. Wofsy, M. B. McElroy, F. W. Valois, and S. W. Watson. 1980. Production of NO_2 and N_2O by Nitrifying Bacteria at Reduced Concentrations of Oxygen. Applied and Environmental Microbiology 40:526-532.

Groffman, P., M. Altabet, J. K. Böhlke, K. Butterbach-Bahl, M. David, M. Firestone, A. Giblin, T. Kana, L. P. Nielsen, and M. Voytek. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16:2091-2122.

Groffman, P. M., E. A. Holland, D. D. Myrold, G. P. Robertson, and X. Zou. 1999. Denitrification. Pages 272-290 in G. P. Robertson, C. S. Bledsoe, D. C. Coleman, and P. Sollins, editors. Standard Soil Methods for Long-Term Ecological Research. Oxford Press, New York.

Harrison-Kirk, T., S. Thomas, T. Clough, M. Beare, T. van der Weerden, and E. Meenken. 2015. Compaction influences N_2O and N_2 emissions from ^{15}N-labeled synthetic urine in wet soils during successive saturation/drainage cycles. Soil Biology and Biochemistry 88:178-188.

Haynes, R. J. 1986. Origin, Distribution and Cycling of Nitrogen in Terrestrial Ecosystems. Page 483 in R. J. Haynes, editor. Academic Press Inc.

Haynes, R. J., and P. H. Williams. 1992. Changes in soil solution composition and pH in urine affected areas of pasture. Journal of Soil Science 43:323-334.

Haynes, R. J., and P. H. Williams. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49:119-199.

He, X., M. Xu, G. Qiu, and J. Zhou. 2009. Use of ^{15}N stable isotope to quantify nitrogen transfer between mycorrhizal plants. Journal of Plant Ecology 2:107-118.

Henry, S., D. Bru, B. Stres, S. Hallet, and L. Philippot. 2006. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Applied and Environmental Microbiology 72:5181-5189.

Hergoualc'h, K., U. Skiba, J. M. Harmand, and R. Oliver. 2007. Processes responsible for the nitrous oxide emission from a Costa Rican Andosol under a coffee agroforestry plantation. Biology and Fertility of Soils 43:787-795.

Hopewell, H. G. 1958. Sprinkler irrigation of pastures in the North Island. New Zealand Journal of Agriculture 96:106-110.

Hopewell, H. G. 1960. Spray irrigation. New Zealand Journal of Agriculture 100:77.

Houlbrooke, D. J., R. P. Littlejohn, J. D. Morton, and R. J. Paton. 2008. Effect of irrigation and grazing animals on soil quality measurements in the North Otago Rolling Downlands of New Zealand. Soil Use and Management 24:416-423.

Jarvis, S. C., and B. F. Pain. 1990. Ammonia volatilization from agricultural land. Proceedings - Fertiliser Society 298:1-35.

Jones, C., B. Stres, M. Rosenquist, and S. Hallin. 2008. Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. Molecular Biology and Evolution 25:1955-1966.

Kester, R. A., W. De Boer, and H. J. Laanbroek. 1997. Production of NO and N_2O by Pure Cultures of Nitrifying and Denitrifying Bacteria during Changes in Aeration. Applied and Environmental Microbiology 63:3872-3877.

Khalil, K., B. Mary, and P. Renault. 2004. Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O_2 concentration. Soil Biology and Biochemistry 36:687-699.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Kool, D., J. Dolfing, N. Wrage, and J. Van Groenigen. 2011a. Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. Soil Biology and Biochemistry 43:174-178.

Kool, D., J. W. Van Groenigen, and N. Wrage. 2011b. Source determination of nitrous oxide based on nitrogen and oxygen isotope tracing dealing with oxygen exchange. Methods in Enzymology 496:139-160.

Kuenen, and L. Robertson. 1994. Combined nitrification-denitrification processes. FEMS Microbiology Reviews 15:109-117.

Kuenen, G. 2008. Anammox bacteria: From discovery to application. Nature Reviews. Microbiology 6:320-326.

Linn, D. M., and J. W. Doran. 1984. Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. Soil Science Society of America Journal 48:1267-1272.

Liu, C., K. Wang, S. Meng, X. Zheng, Z. Zhou, S. Han, D. Chen, and Z. Yang. 2011. Effects of irrigation, fertilization and crop straw management on nitrous oxide and nitric oxide emissions

from a wheat–maize rotation field in northern China. Agriculture, Ecosystems and Environment 140:226-233.

Long, A., J. Heitman, C. Tobias, R. Philips, and B. Song. 2013. Co-occurring anammox, denitrification, and codenitrification in agricultural soils. Applied and Environmental Microbiology 79:168-176.

Ma, L., C. Guo, X. Lü, S. Yuan, and R. Wang. 2015. Soil moisture and land use are major determinants of soil microbial community composition and biomass at a regional scale in northeastern China. Biogeosciences:2585-2596.

MacLeod, C., and H. Moller. 2006. Intensification and diversification of New Zealand agriculture since 1960: An evaluation of current indicators of land use change. Agriculture, Ecosystems and Environment 115:201-218.

Maharjan, B., R. Venterea, and C. Rosen. 2014. Fertilizer and Irrigation Management Effects on Nitrous Oxide Emissions and Nitrate Leaching. Agronomy Journal 106:703-714.

Matheson, F. E., M. L. Nguyen, A. B. Cooper, T. P. Burt, and D. C. Bull. 2002. Fate of ^{15}N-nitrate in unplanted, planted and harvested riparian wetland soil microcosms. Ecological Engineering 19:249-264.

Mathieu, O., C. Hénault, J. Lévêque, E. Baujard, M. J. Milloux, and F. Andreux. 2006. Quantifying the contribution of nitrification and denitrification to the nitrous oxide flux using ^{15}N tracers. Environmental Pollution 144:933-940.

McBride, S. D. 1994. Pasture yield responses to irrigation in Canterbury. Proceedings of the New Zealand Grassland Association 56:165-168.

McDowell, R. W., J. J. Drewry, R. J. Paton, P. L. Carey, R. M. Monaghan, and L. M. Condron. 2003. Influence of soil treading on sediment and phosphorus losses in overland flow. Australian Journal of Soil Research 41:949-961.

Menneer, J., S. Ledgard, C. McLay, and W. Silvester. 2005. Animal treading stimulates denitrification in soil under pasture. Soil Biology and Biochemistry 37:1625-1629.

Ministry for the Environment. 2010. Land use Environmental Snapshot. From http://www.mfe.govt.nz/more/environmental-reporting/reporting-act/land/land-use-indicator/land-use-environmental-snapshot. Site accessed May 30, 2015.

Ministry for the Environment. 2013. New Zealand's Greenhouse Gas Inventory 1990 – 2011. http://www.mfe.govt.nz/publications/climate-change-environmental-reporting/new-zealands-greenhouse-gas-inventory-1990%E2%80%932011. Site accessed May 15, 2015.

Moir, J. L., K. C. Cameron, and H. J. Di. 2007. Effects of the nitrification inhibitor dicyandiamide on soil mineral N, pasture yield, nutrient uptake and pasture quality in a grazed pasture system. Soil Use and Management 23:111-120.

Moir, J. L., K. C. Cameron, H. J. Di, and U. Fertsak. 2011. The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. The Journal of Agricultural Science 149:473-485.

Morley, N., E. Baggs, P. Dörsch, and L. Bakken. 2008. Production of NO, N_2O and N_2 by extracted soil bacteria, regulation by NO_2^- and O_2 concentrations. FEMS Microbiology Ecology 65:102-112.

Morley, N., and E. M. Baggs. 2010. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biology and Biochemistry 42:1864-1871.

Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. van Cleemput. 1998. Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. Nutrient Cycling in Agroecosystems 52:225-248.

Mosier, A. R., W. D. Guenzi, and E. E. Schweizer. 1986. Soil Losses of Dinitrogen and Nitrous Oxide from Irrigated Crops in Northeastern Colorado. Soil Science Society of America Journal 50:344-348.

Müller, C., R. Laughlin, O. Spott, and T. Rütting. 2014. Quantification of N_2O emission pathways via a 15N tracing model. Soil Biology and Biochemistry 72:44-54.

Müller, C., R. J. Stevens, R. J. Laughlin, F. Azam, and J. C. G. Ottow. 2002. The nitrification inhibitor DMPP had no effect on denitrifying enzyme activity. Soil Biology and Biochemistry 34:1825-1827.

New Zealand Government National Infrastructure Unit. 2015. National Infrastructure Plan 2015: The Thirty Year New Zealand Infrastructure Plan. Pages 1-83.

Oenema, O., G. Gebauer, M. Rodriguez, A. Sapek, S. C. Jarvis, W. J. Corré, and S. Yamulki. 1998. Controlling nitrous oxide emissions from grassland livestock production systems. Nutrient Cycling in Agroecosystems 52:141-149.

Orwin, K. H., J. E. Bertram, T. J. Clough, L. M. Condron, R. R. Sherlock, M. O'Callaghan, J. Ray, and D. B. Baird. 2010. Impact of bovine urine deposition on soil microbial activity, biomass, and community structure. Applied Soil Ecology 44:89-100.

Owens, J., T. Clough, J. Laubach, J. Hunt, R. Venterea, and R. Phillips. 2015. Nitrous oxide fluxes, soil oxygen, and denitrification potential from urine and non-urine treated soil under different irrigation frequencies. Journal of Environmental Quality.

Parkin, T. 1987. Soil Microsites as a Source of Denitrification Variability. Soil Science Society of America 51:1194-1199.

Pathak, H. 1999. Emissions of nitrous oxide from soil. Current Science 77:359-369.

Pauleta, S., S. Dell'Acqua, and I. Moura. 2013. Nitrous oxide reductase. Coordination Chemistry Reviews 257:332-349.

Phillips, F. A., R. Leuning, R. Baigent, K. B. Kelly, and O. T. Denmead. 2007. Nitrous oxide flux measurements from an intensively managed irrigated pasture using micrometeorological techniques. Agricultural and Forest Meteorology 143:92-105.

Pihlatie, M., E. Syväsalo, A. Simojoki, M. Esala, and K. Regina. 2004. Contribution of nitrification and denitrification to N_2O production in peat, clay and loamy sand soils under different soil moisture conditions. Nutrient Cycling in Agroecosystems 70:135-141.

Poth, M., and D. D. Focht. 1985. ^{15}N kinetic analysis of N_2O production by Nitrosomonas europaea: An examination of nitrifier denitrification. Applied and Environmental Microbiology 49:1134-1141.

Qu, Z., J. Wang, T. Almøy, and L. Bakken. 2014. Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O+N_2)$ product ratio of denitrification, primarily due to acidification of the soils. Global Change Biology 20:1685-1698.

Ruser, R., H. Flessa, R. Russow, G. Schmidt, F. Buegger, and J. C. Munch. 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. Soil Biology and Biochemistry 38:263-274.

Russow, R., C. F. Stange, and H. U. Neue. 2009. Role of nitrite and nitric oxide in the processes of nitrification and denitrification in soil: Results from ^{15}N tracer experiments. Soil Biology and Biochemistry 41:785-795.

Ryden, J., and K. Dawson. 1982. Evaluation of the acetylene-inhibition technique for the measurement of denitrification in grassland soils. Journal of the Science of Food and Agriculture 33:1197-1206.

Saggar, S., N. S. Bolan, R. Bhandral, C. B. Hedley, and J. Luo. 2004. A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. New Zealand Journal of Agricultural Research 47:513-544.

Sahrawat, K. L. 2008. Factors Affecting Nitrification in Soils. Communications in Soil Science and Plant Analysis 39:1436-1446.

Sainju, U., W. Stevens, T. Caesar-Tonthat, and M. Liebig. 2012. Soil greenhouse gas emissions affected by irrigation, tillage, crop rotation, and nitrogen fertilization. Journal of Environmental Quality 41:1774-1786.

Sanford, R., D. Wagner, Q. Wu, J. Chee-Sanford, S. Thomas, C. Cruz-García, G. Rodríguez, A. Massol-Deyá, K. Krishnani, K. Ritalahti, S. Nissen, K. Konstantinidis, and F. Löffler. 2012. Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. Proceedings of the National Academy of Sciences 109:19709-19714.

Santoro, A. E. 2016. The do-it-all nitrifier. Science 351:342-343.

Saunders, C., and J. Saunders. 2012. The economic value of potential irrigation in Canterbury. Lincoln, Canterbury: Agribusiness and Economics Research Unit (AERU), Lincoln University

Saunders, N. F., J. J. Hornberg, W. N. Reijnders, H. V. Westerhoff, S. de Vries, and R. J. van Spanning. 2000. The NosX and NirX proteins of Paracoccus denitrificans are functional homologues: their role in maturation of nitrous oxide reductase. Journal of Bacteriology 182:5211-5217.

Scheer, C., P. R. Grace, D. W. Rowlings, and J. Payero. 2012. Nitrous oxide emissions from irrigated wheat in Australia: Impact of irrigation management. Plant and Soil 359:351-362.

Scheer, C., P. R. Grace, D. W. Rowlings, and J. Payero. 2013. Soil N_2O and CO_2 emissions from cotton in Australia under varying irrigation management. Nutrient Cycling in Agroecosystems 95:43-56.

Schoeller, D. 1999. Isotope Fractionation: Why Aren't We What We Eat? Journal of Archaeological Science 26:667-673.

Selbie, D., L. Buckthought, and M. Shepherd. 2015a. The Challenge of the Urine Patch for Managing Nitrogen in Grazed Pasture Systems. Advances in Agronomy 129:229-292.

Selbie, D., K. C. Cameron, H. J. Di, J. L. Moir, G. J. Lanigan, and K. G. Richards. 2014. The fate of urine nitrogen: A grassland lysimeter study in Ireland. Pages 1-7 in Nutrient management for the farm, catchment and community, Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Selbie, D. R., G. J. Lanigan, R. J. Laughlin, H. J. Di, J. L. Moir, K. C. Cameron, T. J. Clough, C. J. Watson, J. Grant, C. Somers, and K. G. Richards. 2015b. Confirmation of co-denitrification in grazed grassland. Nature Scientific Reports 5:17361.

Sherlock, R., P. Jewell, and T. Clough. 2011. Review of New Zealand specific FRAC(GASM) and FRAC(GASF) emissions factors. ISSN 2230-2794.

Sherlock, R. R. 1984. Dynamics of ammonia volatilization and nitrous oxide production from urine patches in grazed pastures. [PhD thesis]. Christchurch: Lincoln University.

Sherlock, R. R., and K. M. Goh. 1984. Dynamics of ammonia volatilization from simulated urine patches and aqueous urea applied to pasture I. Field experiments. Fertilizer Research 5:181-195.

Signor, D., C. Eduardo, and P. Cerri. 2013. Nitrous oxide emissions in agricultural soils: A review. Pesquisa Agropecuária Tropical 43.

Silver, W., D. Herman, and M. Firestone. 2001. Dissimilatory Nitrate Reduction to Ammonium in Upland Tropical Forest Soils. Ecology 82:2410-2416.

Šimek, M., and J. E. Cooper. 2002. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. European Journal of Soil Science 53:345-354.

Simojoki, A., and A. Jaakkola. 2000. Effect of nitrogen fertilization, cropping and irrigation on soil air composition and nitrous oxide emission in a loamy clay. European Journal of Soil Science 51:413-424.

Smith, K. A., P. E. Thomson, H. Clayton, I. P. McTaggart, and F. Conen. 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmospheric Environment 32:3301-3309.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko, and C. 2007. Agriculture.in B. Metz, O. R. Davidson, P. R. Bosch, R. Dave, and L. A. Meyer, editors. Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Spott, O., R. Russow, and C. F. Stange. 2011. Formation of hybrid N_2O and hybrid N_2 due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. Soil Biology and Biochemistry 43:1995-2011.

Statistics Zealand. Dairy industry 'mooooving' forward. 2012. Statistics New Zealand Tatauranga Aotearoa, NZ Official Yearbook 2012. From: http://www.stats.govt.nz/browse_for_stats/snapshots-of-nz/yearbook/environment/agriculture/dairy.aspx. Site accessed May 28, 2015

Stevens, R. J., and R. J. Laughlin. 1998. Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. Nutrient Cycling in Agroecosystems 52:131-139.

Stevenson, B., L. Schipper, A. McGill, and D. Clark. 2011. Denitrification and availability of carbon and nitrogen in a well-drained pasture soil amended with particulate organic carbon. Journal of Environmental Quality 40:923-930.

Strohm, T., B. Griffin, W. Zumft, and B. Schink. 2007. Growth Yields in Bacterial Denitrification and Nitrate Ammonification. Applied and Environmental Microbiology 73:1420-1424.

Thom, E. R., V. T. Burggraaf, C. D. Waugh, and D. A. Clark. 2001. Effects of pasture species and irrigation on milk production over four summers in Waikato. Proceedings of the New Zealand Grassland Association 63: 215-221

Thomas, S. M., M. H. Beare, G. S. Francis, H. E. Barlow, and D. I. Hedderley. 2008. Effects of tillage, simulated cattle grazing and soil moisture on N_2O emissions from a winter forage crop. Plant and Soil 309:131-145.

Thomson, A., G. Giannopoulos, J. Pretty, E. Baggs, and D. Richardson. 2012. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. Philosophical Transactions of the Royal Society B 367:1157-1168.

Tiedje, J. M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. Pages 179-244 in A. J. B. Zehnder, editor. Environmental Microbiology of Anaerobes. John Wiley and Sons.

Trost, B., A. Prochnow, K. Drastig, A. Meyer-Aurich, F. Ellmer, and M. Baumecker. 2013. Irrigation, soil organic carbon and N_2O emissions. A review. Agronomy for Sustainable Development 33:733-749.

Uchida, Y., T. Clough, F. Kelliher, and R. Sherlock. 2008. Effects of aggregate size, soil compaction, and bovine urine on N_2O emissions from a pasture soil. Soil Biology and Biochemistry 40:924-931.

van Cleemput, O. 1998. Subsoils: chemo-and biological denitrification, N_2O and N_2 emissions. Nutrient Cycling in Agroecosystems 52:187-194.

Van Cleemput, O., and A. Samater. 1995. Nitrite in soils: accumulation and role in the formation of gaseous N compounds. Fertilizer Research 45:81-89.

van Groenigen, J., P. Kuikman, W. de Groot, and G. Velthof. 2005. Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. Soil Biology and Biochemistry 37:463-473.

Venterea, R. 2007. Nitrite-driven nitrous oxide production under aerobic soil conditions: kinetics and biochemical controls. Global Change Biology 13:1798-1809.

Venterea, R., and D. Rolston. 2000. Mechanisms and kinetics of nitric and nitrous oxide production during nitrification in agricultural soil. Global Change Biology 6:303-316.

Venterea, R. T., T. J. Clough, J. A. Coulter, F. Breuillin-Sessoms, P. Wang, and M. J. Sadowsky. 2015. Ammonium sorption and ammonia inhibition of nitrite-oxidizing bacteria explain contrasting soil N_2O production. Scientific Reports 5:12153.

Ward, B. B. 2013. Nitrification. Reference Module in Earth Systems and Environmental Sciences. Elsevier.

Whitehead, D. C. 1995. Grassland Nitrogen. CAB International, Wallingford, Oxon, UK.

Whitehead, D. C., and A. W. Bristow. 1990. Transformations of Nitrogen Following the Application of 15N-Labelled Cattle Urine to an Established Grass Sward. Journal of Applied Ecology 27:667-678.

Whittaker, M., D. Bergmann, D. Arciero, and A. Hooper. 2000. Electron transfer during the oxidation of ammonia by the chemolithotrophic bacterium Nitrosomonas europaea. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1459:346-355.

Wrage, N., G. L. Velthof, H. J. Laanbroek, and O. Oenema. 2004. Nitrous oxide production in grassland soils: assessing the contribution of nitrifier denitrification. Soil Biology and Biochemistry 36:229-236.

Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry 33:1723-1732.

Wu, D., W. Dong, O. Oenema, Y. Wang, I. Trebs, and C. Hu. 2013. N_2O consumption by low-nitrogen soil and its regulation by water and oxygen. Soil Biology and Biochemistry 60:165-172.

Yoshinari, T., and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications 69:705-710.

Zhu, X., M. Burger, T. Doane, and W. Horwath. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proceedings of the National Academy of Sciences 110:6328-6333.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61:533-616.

Chapter 3. General Methods

3.1 Soil Properties and Soil Water

Soil bulk density (ρb, Mg m^{-3}) was calculated as:

$$\rho_b = M_s/V_s \quad \text{Equation 3.1}$$

Where Ms is the mass of dry soil (Mg), and *Vs* is the volume of soil core (m^3).

Total porosity (ϕ, %) was determined as:

$$\Phi = \left(1 - \left(\frac{\rho_b}{\rho_d}\right)\right) * 100 \quad \text{Equation 3.2}$$

Where ρd is the particle density, which is assumed to be 2.65 Mg m^{-3}.

Gravimetric soil moisture (θg) was determined by oven drying soil subsamples at 105°C for 24 h (Blakemore et al. 1987). Gravimetric soil moisture (θg, Mg) was calculated as:

$$\theta g = M_{SW}/M_S \quad \text{Equation 3.3}$$

Where M_{sw} is the mass of the water in the soil (Mg).

Volumetric water content (θv, m^3 m^{-3}*)* was determined by:

$$\theta_v = \theta_g * (\frac{\rho_b}{\rho_d}) \quad \text{Equation 3.4}$$

Water-filled pore space (WFPS, m^3 m^{-3}) was calculated as:

$$WFPS = \frac{\theta v}{\Phi} \quad \text{Equation 3.5}$$

Air-filled pore space (ε, m^3 m^{-3}) was calculated as:

$$\boldsymbol{\varepsilon} = \Phi - \theta v \quad \text{Equation 3.6}$$

3.2 Repacking Soil Cores

The re-packed soil cores used soil sieved to > 2 mm. To determine the amount of dry soil needed required to achieve the desired soil ρb (Mg m^{-3}):

$$M_s = \rho_b * V_s + \theta_g * \rho_b * V \quad \text{Equation 3.7}$$

Where *Vs* is the volume of the soil core section (m^3).

A fixed amount of water was then added to the soil prior to packing with the exact amount dependent on the resulting soil ρb. Soil cores were then prepared by packing the soil to a known depth into stainless steel cylinders (73 mm i.d., 71 mm total depth deep). The soil cores were repacked to different depths, depending on the experiment. To obtain uniform bulk densities, soil was compressed into the cores in four stages, 10 mm depth at a time (Figure 3.1).

Figure 3.1 The sections for repacking the soil cores (front) and the stainless steel core (back).

Bulk density was checked after re-packing by randomly selecting a subset of cores and oven drying soil at 105°C for 24 h.

3.3 Soil Chemical Data

The soil chemical data are described in Chapter 4 and Chapter 5, which are written as manuscripts. The soil chemical data information is for reference in Chapter 6, Chapter 7, and Chapter 8.

Ancillary soil chemical data were determined from sub-samples. Soil pH was determined by mixing soil subsamples with deionized (DI) water at a 10 g dry weight: 25 mL DI water ratio (Blakemore et al. 1987), which was measured after 12 h on a pH meter (SevenEasy, Mettler Toledo, Port

Melbourne, Australia). Electrical conductivity was determined by combining 10 g dry weight equivalent of soil with 50 mL of DI water, mixing for 30 min and measuring (SevenEasy, Mettler Toledo, Port Melbourne, Australia) following 5 min of centrifuging at 1500 rpm (Blakemore et al. 1987). Inorganic N concentrations - nitrate (NO_3^--N) and ammonium (NH_4^+-N) - were determined by extracting soil subsamples with 2M KCl at a 4 g dry weight equivalent: 40 mL KCl ratio. Samples were mixed on an end-over-end shaker for 1 h followed by 20 min of centrifuging at 2000 rpm before gravity filtering through Whatman °42 filters (Blakemore et al. 1987). Extracts were frozen until flow injection analyses for NO_3^--N and NH_4^+-N (FIAstar 5000 Analyzer, FOSS Analytical, Hilerød, Denmark).

Hot water carbon (HWC), which is indicative of microbial biomass, and cold water carbon (CWC), which is indicative of the water soluble or leachable carbon, were determined using water extractions (Ghani et al. 2003). Soil and DI water were combined at a 3 g dry weight equivalent: 30 mL DI ratio and mixed on an end-over-end shaker for 30 min and centrifuged at 3500 rpm followed by filtering through Avantec 5C to obtain CWC extracts (Ghani et al. 2003). Once filtered, the soil was re-extracted a second time with hot water to obtain the HWC extraction. After adding DI water as before, the soil-DI water mixture was placed in a hot water bath at 80°C for 16 h before mixing, centrifuging, and filtering using the same process as the CWC (Ghani et al. 2003). The CWC and HWC samples were frozen after extraction until analysis on a Total Organic Carbon Analyser (TOC 5000A, Shimadzu, Australia).

3.4 Nitrous Oxide Gas and Isotopic Sample Analysis

Stable isotope gas samples were analysed using an isotope ratio mass spectrometer, and were taken in Experiment 4, Chapter 7. Samples of N_2O concentrations were taken during field sampling campaigns (Experiments 1 and 2) and during one lab experiment (Experiment 4). Field gas sampling methods used in Experiment 1 and 2 are described in section 3.4.3. Gas sampling during denitrification enzymes assays are discussed in section 3.5 "Denitrification Enzyme Assays".

3.4.1 Isotope Ratio Mass Spectrometer

An isotope ratio mass spectrometer (PDZ Europa Ltd, Crewe, UK) interfaced to a TGII cryfocusing unit (PDZ-Europa Ltd 20-20) was used to determine the ^{15}N enrichment of N_2O and N_2 in gas samples (Stevens and Laughlin 1998). Each gas sample (15 mL) was taken using a gas-tight syringe after the headspace of the jar in which the soil core was placed, had been sealed for 3 h and was

then transferred into a pre-evacuated 12 mL vial (Exetainer® tubes, Labco Ltd, UK). Fluxes of N_2 were calculated using the equations for triple collector mass spectrometers (Mulvaney and Boast 1986).

3.4.2 N_2O by Gas Chromatography

Nitrous oxide fluxes were determined using an automated GC (8610, SRI Instruments, Torrance, CA) interfaced to an autosampler (Gilson 222XL, Middleton, WI) as described by Clough et al. (2006). The autosampler had a purpose-built double concentric injection needle (PDZ Europa Ltd, Crewe, UK) which enabled rapid transfer of the entire gas sample from the Exetainer into the GC. The GC had two 3 mm OD SS columns; a 1 m long pre-column preceded a 6 m long analytical column, both packed with Haysep Q. An automated 10-port gas sampling valve on the GC sent the O_2-free N_2 carrier gas (40 ml min^{-1}) through both pre-column and analytical column in either 'inject' or 'back flush' modes. A 4-port gas sampling valve attached at the posterior of the analytical column was synchronized to send the gas stream to the detector.

3.4.3 Field Sampling of Nitrous Oxide

3.4.3.1 Chamber Description

Soil-to-atmosphere N_2O fluxes were measured using vented insulated non-steady state chambers (headspace volume = 19.625 L) following standardized protocols (Parkin et al. 2012). Chamber tops were insulated with styrofoam to minimize temperature differences between inside and outside of chamber. The vents of the chambers were removed before the chamber lids were set on stainless-steel bases, and were replaced when the lids were in place. To seal chambers during sampling, annular moats on the bases were filled with water. The bases for the chambers were inserted 100 mm in to the soil.

3.4.3.2 Field Gas Sampling Strategy

Gas sampling at time zero (T0) for each chamber occurred immediately after the chamber lid was in place and the vents were secured. Successive samples were taken at 15 minute intervals for 45 minutes for a total of four N_2O gas concentrations samples were available for calculating gas fluxes as changers in concentrations. In rare instances where a sample were discarded (due to improperly secured lid, improper vial evacuation, etc.) three samples were used to calculate the N_2O fluxes.

For each field trial, sampling occurred one day before the urine treatment application, and on the day that the urine was applied (day 0 of the experiment), the gas sampling occurred before the urine was applied. Gas samples each chamber using a glass syringe (20 mL) fitted with a 3-way stopcock, and immediately transferred to 6 mL pre- evacuated (-1 atm) glass Exetainers® (Labco Ltd., Lampeter, United Kingdom). Gas samples were analyzed on an automated SRI gas chromatograph (GC) system equipped with an electron capture detector (SRI 8610c GC, SRI Instruments, Torrance, California, USA) as described in Clough et al. (1996) and in section 3.4.2.

3.4.3.3 Nitrous Oxide Flux Calucations and Corrections

Fluxes in the field were measured between 10:00 am and 12:00 pm to be representative of daily N_2O fluxes (van der Weerden et al. 2013). Field N_2O fluxes calculations used the ideal gas law, and used air temperature, chamber volume and area, and the change in N_2O concentration over time, to calculate the fluxes. Regression of parts per million (ppm) concentrations over the time that the chambers lids were deployed resulted in a slope with units of µL gas L^{-1} h^{-1}. Multiplying the slope by the chamber volume (L) and dividing by the chamber surface area (m^2) resulted in a flux with units of µL trace gas m^{-2} min^{-1}. If the rate of change of headspace trace gas concentration is not constant (i.e. ppm vs. time data was not linear), then a quadratic regression (QR) (Wagner et al. 1997) was used instead of a linear regression (LR). A quadratic regression may be necessary when gas build up occurs in the chamber headspace altered the diffusion gradient and thus the flux of gas from the soil to the atmosphere (Hutchinson and Mosier, 1981). The QR flux was selected for the N_2O flux calculations unless the second derivative of the regression model was ≥ 0 (Venterea et al. 2009, Venterea 2013) according to the LINEST function in Microsoft Excel (version 2013).

The static chamber methods can create bias due to the accumulation of gases in the headspace and the soil (Venterea and Baker, 2008) from both the LR and the QR flux determination methods (Livingston et al., 2006; Venterea et al., 2009). The extent of the bias increases with higher soil air-filled porosity. To account for such a bias in the fluxes, a correction was applied which uses soil bulk density, soil water content, soil texture, and temperature at the time of flux-measurement (Venterea, 2010). For all field gas fluxes, this correction factor was applied to account for chamber-induced artifacts.

Fluxes below the detection limit (Parkin et al. 2012) were assigned a value of zero. The detection limit coefficients were determined using Monte Carlo simulations over a range of analytical precisions and chamber deployment times. Both positive and negative detection limits were

determined using the coefficients, and differed based on the number of samples and the method of flux determination used (either LR or QR).

3.5 Denitrification Enzyme Assays

Denitrification potential measurements do not compare well to actual field fluxes (Groffman 1987, Martin et al. 1988) because they represent "optimal" nutrient and soil O_2 conditions for denitrification which may not occur in the field.

Denitrification potential was determined using denitrification enzyme assays (DEA) following the protocol outlined in Drury et al. (2008). Briefly, the DEA involved mixing 25 mL of a solution containing 50 µg g^{-1} of NO_3^--N (as KNO_3) and 300 µg g^{-1} of C as hot water carbon (HWC) as the C source with 20 g dry weight equivalent (~25 g field moist soil) of soil. The substrate solution and soil were mixed in a 250 mL Mason jar with a gas-tight lid fitted with a rubber septum. The HWC was extracted from the same soil used for the DEAs using the HWC extraction method described in section 3.3 (Ghani et al. 2003). The sealed jars were agitated and flushed continuously with N_2 (instrument grade, <0.001% O_2) for at least 10 min to create anaerobic conditions (Groffman et al. 2006). Acetylene (C_2H_2, instrument grade >98%, < 2% air), purified using an activated charcoal filter (Matheson Gas Products Gas Purifier Model 450B), was added to the headspace at a 10% vol vol^{-1} concentration to inhibit N_2OR, making N_2O the end product of denitrification (Yoshinari and Knowles 1976). Half of the jars had acetylene gas added to the headspace (+C_2H_2 to determine DEA-N_2O+N_2) and the other half of the jars did not (-C_2H_2 to determine DEA-N_2O). All of the jars were incubated at 20°C for a total of 48 h. The jars were re-flushed with N_2 for 10 min prior to each instance of sampling for N_2O concentrations. After sampling, C_2H_2 was re-added to the +C_2H_2 samples (10% vol vol^{-1}). Each jar was sampled for five min at one min intervals during sampling to estimate N_2O fluxes (Figure 3.3).

3.5.1 Closed Sampling System

To sample N_2O concentrations during DEA's, three 16 gauge needles connected to two-way stop cock valves that were inserted into the septa of the jars lid, each connecting to silicon tubing. The needle tips were injected into the septa of the jars. One of the three lines connected the pressurized N_2 gas container to the jar, and a secondary line was created from this same valve and connected to a 250 mL Erlenmeyer flask filled with water. This output apparatus was used to ensure that the jars were not over-pressurized. After the N_2 gas was turned off, the secondary

valve was opened, and closed when the bubbling stopped indicating pressure equilibration. Another line connected the jar to the input of a photo-acoustic analyzer (Brüel and Kjaer, Multi-gas monitor type 1302, Denmark). This line also included a ~20 mL filter inline filled with a self-indicating CO_2 and moisture scrub (Carbozorb AS, BDH Chemicals Ltd., Rool, England) which was positioned vertically to allow moisture to drip to the bottom. A third line connected the jar to the outlet of the photo-acoustic analyzer. During N_2 flushing, this line was closed to the jar and open to the atmosphere, providing an exhaust for the system. During gas sampling, this line was closed to the atmosphere and open to the jar, when the N_2 input line was closed to the jar, as was the inline valve connecting the input line and the flow meter, creating a "closed" system (Figure 3.3). When the system was closed, there was no input or output to the atmosphere, and the photo-acoustic analyzer pump, which draws air in from the jar via the "in line" and out via the "output line" during sampling, which is was responsible for circulating the gas in the system. When the analyzer was not sampling, the gas was not circulating through the closed system.

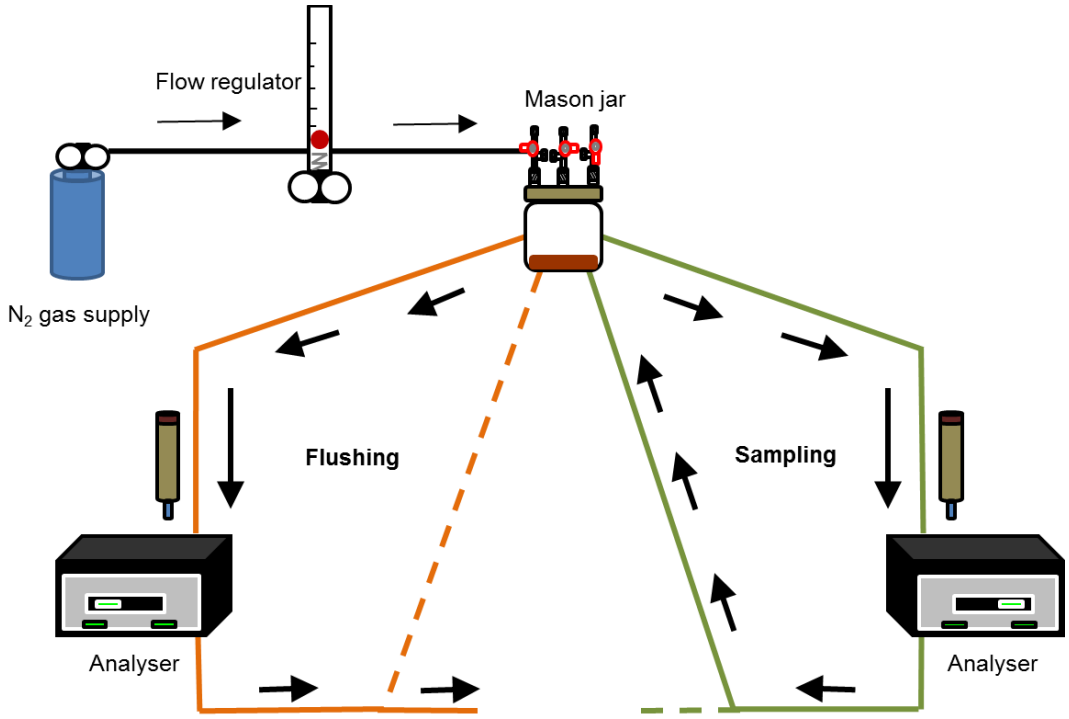


Figure 3.2 A diagram showing the setup of the photoacoustic analyzer with the left hand side showing the set up while flushing (orange) and the right hand side showing the closed loop set up while sampling (green). The dotted line indicates no flow.

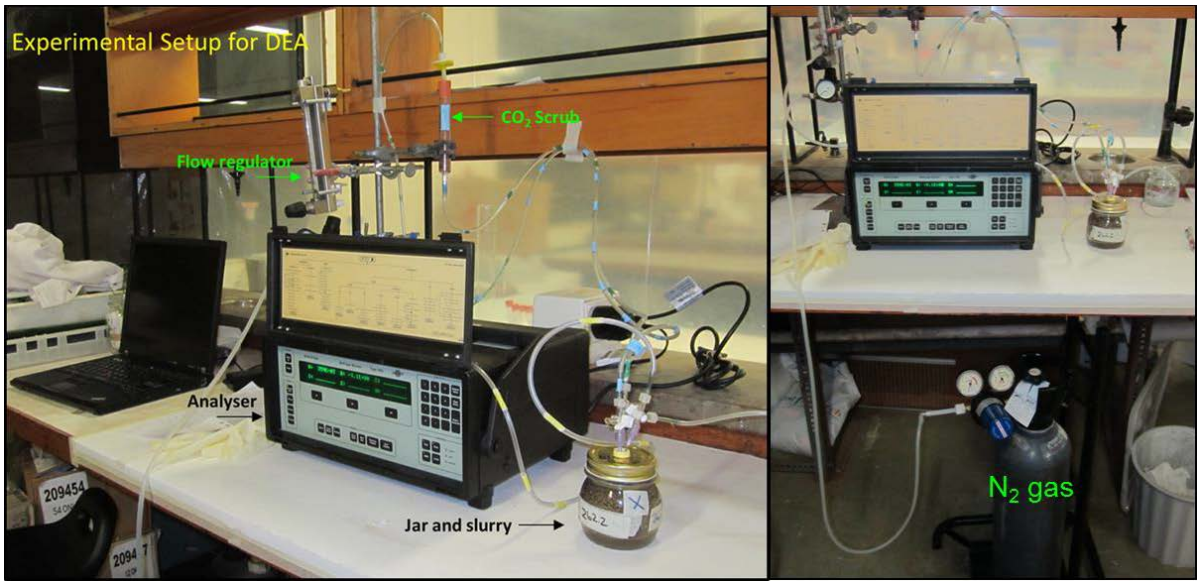


Figure 3.3 The setup for the denitrification enzyme assay with the left showing the system set up, and the right showing the N_2 gas source used for flushing

3.5.2 Denitrification Enzyme Assays Calculations

Nitrous oxide flux calculations were performed as outlined in Drury et al. (2008), making allowances for N_2O dissolved in the soil water using the Bunsen absorption coefficient (Tiedje 1982). Total N_2O evolved over each 48 h incubation period represented either the DEA-N_2O+N_2 (from the +C_2H_2 samples) or the DEA-N_2O (N_2O from the -C_2H_2 samples) which were then expressed as the DEA-N_2O/(DEA-N_2O+N_2) ratio.

The amount of N_2O evolved over the 48 hour incubation period was determined using the ideal gas law, correcting for dissolved N_2O in solution using the Bunsen absorption coefficient (α), which was taken to be 0.632 mL N_2O mL^{-1} water at 20°C (Tiedje 1982). At time t, the volume of N_2O evolved (V_{N2Ot}, µL) was calculated as such:

$$V_{N2Ot} = (Ct[V_h * (V_{water} * \ \alpha)]) * \left(\frac{1L}{1000mL}\right) \qquad \text{Equation 3.8}$$

Where Ct (µL N_2O L^{-1}) is the N_2O gas concentration in the gas phase at time t,

Vh (mL) is the volume of the headspace,

V_{water} (mL) is the volume of water in the soil during the incubation, and

α (mL N_2O mL^{-1} water) is the Bunsen absorption coefficient.

Using the ideal gas law, the concentration of N_2O-N is calculated:

$$N_2O - Nt = \frac{VN_2Ot * P\left(28.0134\ g\ N_2O{-}N\ mol^{-1}\right)}{R * T * M_S} \qquad \text{Equation 3.9}$$

Where N_2O-Nt (µg N_2O-N g^{-1}) is the concentration of N_2O-N at time t,

VN_2Ot (µL) is the volume of N_2O in the jar at time t,

P is the pressure in kPa,

R is the universal gas constant (8.31451 L kPa mol^{-1} K^{-1}),

T is temperature in K, and

M_S is the oven-dry mass of soil (g)

A linear regression was performed for each accumulation period, and a *P* value of 0.05 was used as the threshold for significance. When the regression was found to not be significant, a value of zero was attributed to the fluxes.

The cumulative N_2O evolved over the 48 hour incubation period was expressed as the denitrification potential, unless otherwise specified.

The volume of each Mason jar was calculate by:

$$V_h = V_{total} - (V_{soil} + V_{water}) \qquad \text{Equation 3.10}$$

Where V_h is the total volume of the jar (mL),

V_{soil} volume of soil in the jar (mL), and

V_{water} volume of the water in the jar (mL).

To get V_{soil}, the dry weight equivalent of the soil (Mg) is divided by the assumed ρd (2.65 Mg m^{-3}).

The V_{water} was determined by multiplying the amount of water gravimetrically as a percentage (i.e. 30% = 0.3) by the amount of dry soil (Mg), i.e. (0.3 * 20000 Mg = 6.00 mL water present in the soil by volume, assuming a water density of 1000 Mg mL).

Because gas sampling was completed using a closed loop system using a photo-acoustic analyzer, there was no need to adjust the headspace pressure due to extraction of the gas samples.

Compensating for Acetylene Addition

The ideal gas law was used to account for the pressure change resulting from adding C_2H_2 to the system, and the amount of gas that must be removed prior to the addition of C_2H_2 so as to not change the pressure in the system:

$$V_S = \left[\frac{C}{100}\right] * VF \qquad \text{Equation 3.11}$$

Where V_s (mL) is the required syringe sample volume removed from the system,

C (%) is the desired percentage of jar headspace volume to be collected, and

VF (mL) is the headspace volume of the jar.

3.5.2.1 Laboratory Methods N_2O Flux Sampling

For determining N_2O and CO_2 fluxes from soil cores in the laboratory, soil cores were placed in glass mason jars. Gas samples were taken every 15 min for 45 min (Figure 7.4) for a total of four samples per incubation. Samples were collected with a glass syringe and analysed on the GC (section 3.4.2). Fluxes were calculated using the same methods described above in Equation 3.12, Equation 3.13, and Equation 3.14.

3.6 Relative Soil Gas Diffusion

To measure relative soil gas diffusivity (D_P/D_O), at 20°C, an air-tight chamber was purged with a gas mixture (90% Ar and 10% N_2) until O_2 free. The O_2 concentration within the chamber was measured using an O_2 sensor (KE-25, Figaro Engineering Inc., Osaka, Japan). Following the creation of the O_2 free environment in the chamber cavity, the chamber was open only to the soil core, thereby making diffusion through the soil core the only pathway for gas exchange between the atmosphere and the chamber cavity (Figure 3.4). Over 180 min, increases in O_2 measured by the sensor in the chamber cavity were representative of the rate of O_2 diffusion through the soil core, with production or uptake of O_2 by the soil core assumed to be negligible (Moldrup et al. 2000). The slope of the soil O_2 concentrations over time was used to determine D_P (Rolston and Moldrup

2002) while the O_2 diffusion coefficient in free air at 20°C, D_O, was taken to be 0.074 $m^2\ h^{-1}$ (Currie 1960).

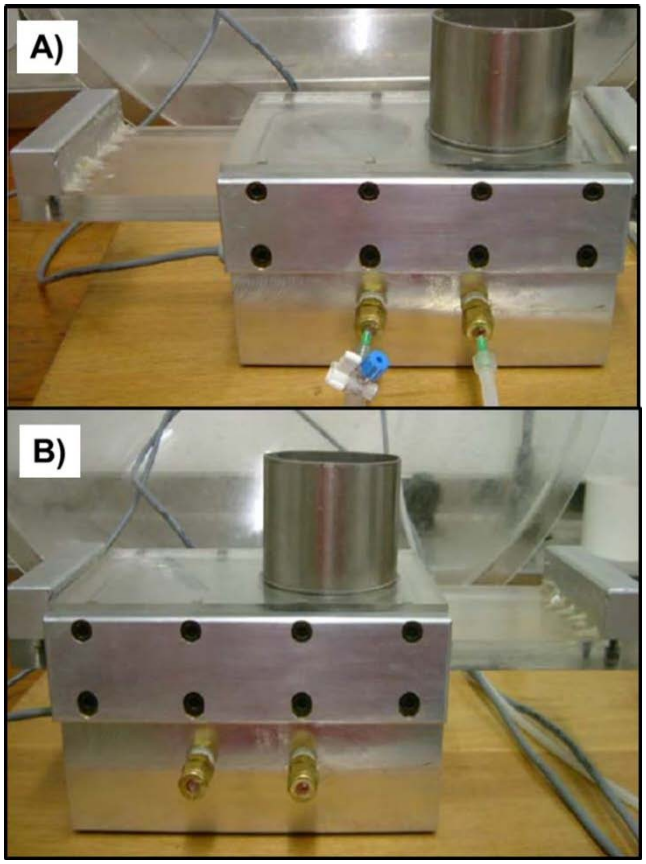


Figure 3.4 The diffusion chamber apparatus and set up for measuring relative soil gas diffusivity when (A) the chamber is closed and being flushed with O_2 free-N_2, and (B) the chamber is open allowing O_2 to diffuse through the soil into the chamber to be measured. (Photo taken by Nimlesh Balaine.)

3.7 Modelling Relative Soil Gas Diffusivity

Relative soil gas diffusivity was modelled using the Structure-dependent Water-induced Linear Reduction (SWLR) model, as defined by Moldrup et al. (2013a). The SWLR model is:

$$\frac{D_P}{D_O} = \varepsilon^{[1+Cm\Phi]}\left(\frac{\varepsilon}{\Phi}\right) \qquad \text{Equation 3.15}$$

The *Cm* is media complexity factor, assumed to be related to soil particle density and therefore bulk density. For repacked soil cores, the Cm is 1, and Cm for soils with plants is 2.1 (Moldrup et al. 2013a).

3.8 Data Analysis and Statistics

The data analysis and statistical methods used for each experiment are noted in individual chapters. Parametric statistics were used for analysis throughout the thesis, so data is often transformed to meet the assumption of normality. The transformations are noted in supplementary data.

3.9 References

Blakemore, L. C., P. L. Searle, and B. K. Daly. 1987. Methods for chemical analysis of soils. Pages 7-29 NZ Soil Bureau Scientific Report.

Currie, J. A. 1960. Gaseous diffusion in porous media. Part 1. A non-steady state method. British Journal of Applied Physics 11.

Clough, T.J., Sherlock, R.R., Cameron, K.C., Ledgard, S.F., 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant and Soil, 178, 141-152.

Drury, C. F., D. D. Myrold, E. G. Beauchamp, and W. D. Reynolds. 2008. Denitrification techniques for soil. Pages 471-493 in M. R. Carter and E. G. Gregorich, editors. Soil Sampling and Methods of Analysis. CRC Press, Boca Raton, FL, USA.

Ghani, A., M. Dexter, and K. W. Perrott. 2003. Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology and Biochemistry 35:1231-1243.

Groffman, P. 1987. Nitrification and denitrification in soil: A comparison of enzyme assay, incubation and enumeration methods. Plant and Soil 97:445-450.

Groffman, P., M. Altabet, J. K. Böhlke, K. Butterbach-Bahl, M. David, M. Firestone, A. Giblin, T. Kana, L. P. Nielsen, and M. Voytek. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16:2091-2122.

Hutchinson, G.L., Mosier, A.R., 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Science Society of America Journal, 45, 311-316.

Livingston, G.P., and G.L. Hutchinson. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error. In. P.A. Matson and R.C. Harriss (eds.) Biogenic Trace Gases: Measuring Emissions from Soil and Water. Methods in Ecology. Blackwell Science Cambridge University Press. pp 14-51.

Martin, K., L. L. Parsons, R. E. Murray, and M. S. Smith. 1988. Dynamics of Soil Denitrifier Populations: Relationships between Enzyme Activity, Most-Probable-Number Counts, and Actual N Gas Loss. Applied and Environmental Microbiology 54:2711-2716.

Moldrup, P., T. K. K. Chamindu Deepagoda, S. Hamamoto, T. Komatsu, K. Kawamoto, D. E. Rolston, and L. Wollesen de Jonge. 2013. Structure-Dependent Water-Induced Linear Reduction Model for Predicting Gas Diffusivity and Tortuosity in Repacked and Intact Soil. Vadose Zone Journal 12:2-11.

Moldrup, P., T. Olesen, J. Gamst, P. Schjonning, T. Yamaguchi, and D. Rolston. 2000. Predicting the gas diffusion coefficient in repacked soil: Water-induced linear reduction model. Soil Science Society of America Journal 64:1588-1594.

Mulvaney, R. L., and C. W. Boast. 1986. Equations for Determination of Nitrogen^{-15} Labeled Dinitrogen and Nitrous Oxide by Mass Spectrometry. Soil Science Society of America Journal 50.

Parkin, T. B., R. T. Venterea, and S. K. Hargreaves. 2012. Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. Journal of Environmental Quality 41:705-715.

Rolston, D. E., and P. Moldrup. 2002. Gas diffusivity. Pages 1113-1139 in J. H. Dane and G. C. Topp, editors. Methods of Soil Analysis, part 4, Physical Methods. Soil Science Society of America, Madison, Wisconsin.

Stevens, R. J., and R. J. Laughlin. 1998. Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. Nutrient Cycling in Agroecosystems 52:131-139.

Tiedje, J. M. 1982. Denitrification. Pages 1011-1026 in A. L. Page, R. H. Miller, and D. R. Keeney, editors. Methods of Soil Analysis, Part 2. Agronomy Monograph No. 9.

van der Weerden, T. J., T. J. Clough, and T. M. Styles. 2013. Using near-continuous measurements of N_2O emission from urine-affected soil to guide manual gas sampling regimes. New Zealand Journal of Agricultural Research 56:60-76.

Venterea, R. 2010. Simplified method for quantifying theoretical underestimation of chamber-based trace gas fluxes. Journal of Environmental Quality 39:126-135.

Venterea, R. 2013. Theoretical Comparison of Advanced Methods for Calculating Nitrous Oxide Fluxes using Non-steady State Chambers. Soil Science Society of America Journal 77:709-720.

Venterea, R.T. and J.M. Baker. 2008. Effects of soil physical nonuniformity on chamber-based gas flux estimates. Soil Science Society of America Journal 72:1410-1417.

Venterea, R., K. Spokas, and J. Baker. 2009. Accuracy and Precision Analysis of Chamber-Based Nitrous Oxide Gas Flux Estimates. Soil Science Society of America Journal 73:1087-1093.

Wagner, S. W., D. C. Reicosky, and R. S. Alessi. 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal 89:279-284.

Yoshinari, T., and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications 69:705-710.

Chapter 4.

Nitrous Oxide Fluxes, Soil Oxygen, and Denitrification Potential of Urine- and Non-Urine Treated Soil Under Different Irrigation Frequencies

As noted above, this work has been accepted by the Journal of Environmental Quality as:

Owens, J., Clough, T. J., Laubach, J., Hunt, J. E., Venterea, R. T., and Phillips, R. L. (2015). Nitrous oxide fluxes, soil oxygen, and denitrification potential from urine and non-urine treated soil under different irrigation frequencies. *Journal of Environmental Quality*. DOI: 10.2134/jeq2015.10.0516

Jen Owens, Tim J. Clough, Johannes Laubach, John E. Hunt, Rodney T. Venterea, Rebecca L. Phillips

J. Owens, T. J. Clough, Department of Soil and Physical Sciences, Lincoln University, P.O. Box 85084, Lincoln 7647, New Zealand

J. Laubach, J. E. Hunt, R. L. Phillips, Landcare Research, PO Box 69040, Lincoln 7640, New Zealand

R.T. Venterea, USDA-ARS, Soil and Water Management Research Unit, & Department of Soil and Water, and Climate, Univ. of Minnesota, St. Paul, MN, USA.

Abbreviations list

WFPS, water-filled pore space; D_P/D_O, relative soil gas diffusivity; N_2OR, N_2O reductase enzyme; 3-d, 3-day irrigation regime; 6-d, 6 day irrigation regime; DEA-N_2O/(DEA-N_2O+N_2), ratio of $N_2O/(N_2O+N_2)$ obtained from the denitrification enzyme assays

4.1 Abstract

Despite increased use of irrigation to improve forage quality and quantity for grazing cattle (*Bos taurus,* Linnaeus), there is a lack of data that assess how irrigation practices influence nitrous oxide (N_2O) emissions from urine-affected soils. Irrigation effects on soil oxygen (O_2) availability, a primary controller of N_2O fluxes, is poorly understood. It was hypothesized that increased irrigation frequency would result in lower N_2O emissions by increasing soil moisture and decreasing soil O_2 concentrations. This would favor more N_2O reduction to dinitrogen (N_2). We examined effects of high (3-d) versus low (6-d) irrigation frequency with and without bovine urine addition to pasture. Nitrous oxide fluxes were measured daily for 35 d. Soil O_2, temperature, and water content were continuously measured at multiple depths. Inorganic nitrogen, organic carbon, and soil pH were measured at 6-d intervals. Measurements of denitrification enzyme activity with and without acetylene inhibition were used to infer the $N_2O/(N_2O+N_2)$ ratio. The $N_2O/(N_2O+N_2)$ ratio was lower under high- compared with low-frequency irrigation, suggesting greater potential for N_2O reduction to N_2 with more frequent irrigation. Although N_2O fluxes were increased by urine addition, they were not affected by irrigation frequency. Soil O_2 decreased temporarily after urine deposition, but O_2 dynamics did not explain N_2O dynamics. Relative soil gas diffusivity (D_P/D_O) was a better predictor of N_2O fluxes than O_2 concentration. On a free-draining soil, increasing irrigation frequency while providing the same total water volume did not enhance N_2O emissions under ruminant urine patches in a grazed pasture.

4.2 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) and is the dominant ozone-depleting substance currently emitted (Ravishankara et al. 2009). Agricultural soils are the primary source of anthropogenic N_2O (IPCC 2007) due to nitrogen (N) inputs from fertilizer application and animal excreta (Davidson 2009), especially ruminant urine (Oenema et al. 2005). Upward of 300 million ha of the world's agricultural soils receive irrigation (Food and Agriculture Organization of the United 2010), which helps provide food security but may also alter soil N cycling, thereby affecting N_2O emissions (Trost et al. 2013).

Irrigation improves forage quality and quantity in grazed pastures (McBride 1994), where annual spatial coverage of urine patches can reach ~20% of a paddock (Moir et al. 2011). Few studies have examined how irrigation affects N_2O emissions from urine patches (Di and Cameron 2002b).

Irrigation studies on cropped systems have reported conflicting results; irrigation either increases or has no effect on N_2O emissions (Simojoki and Jaakkola 2000, Horváth et al. 2010, Scheer et al. 2013, Maharjan et al. 2014).

Irrigation may decrease soil oxygen (O_2) concentrations by increasing soil moisture (Trost et al. 2013). Soil O_2 is a proximal controller of biological pathways producing N_2O (Firestone and Davidson 1989). Anaerobic conditions promote N_2O reductase enzyme (N_2OR) activity, which reduces N_2O to dinitrogen (N_2) during denitrification (Knowles 1982). The degree of anaerobiosis determines the relative ratio of N_2O to N_2 emitted (Knowles 1982, Wrage et al. 2001, Zhu et al. 2013). *In situ* soil O_2 concentrations in pastures have never been intensively measured, with only sporadic measurements available (Eccles et al. 1990, Simojoki and Jaakkola 2000). It is unknown how soil O_2 in pastures changes under different irrigation regimes, and such data may help elucidate controls over N_2O fluxes and potential N_2OR activity.

Measures of soil moisture content, such as water-filled pore space (WFPS), are generally used as a proxy for soil O_2–N_2O flux variation (Dobbie et al. 1999, Ruser et al. 2006). However, the WFPS calculation (Linn and Doran 1984) fails to account for pore connectivity and tortuosity (Farquharson and Baldock 2008), which are key factors determining soil gas transport. Relative soil gas diffusivity, D_P/D_O, which is the ratio of the soil–gas diffusion coefficient to the free-air gas diffusion coefficient (Moldrup et al. 2013a), incorporates these factors. It describes the ease of movement of gases through the soil profile and the exchange of gases between the soil and the atmosphere by accounting for the total porosity and air-filled porosity (Moldrup et al. 2013a). Relative soil gas diffusivity has been shown to explain the variability in N_2O emissions in a controlled lab study using repacked cores (Balaine et al. 2013) and from intact soil cores from different cropping systems (Petersen et al. 2013).

This study aimed to quantify the effect of two irrigation frequencies on urine-affected pasture soil with respect to (i) the timing and magnitude of N_2O emissions, (ii) soil O_2 concentrations through direct measurements and estimates of soil D_P/D_O, and (iii) the potential $N_2O/(N_2O + N_2)$ ratio, which is indicative of potential N_2OR. It was hypothesized that more frequent irrigation would keep soil moisture higher, reducing soil O_2 concentrations and thereby promoting N_2OR, leading to a lower $N_2O/(N_2O + N_2)$ ratio and to lower total N_2O emissions.

4.3 Materials and Methods

4.3.1 Study Site

The experiment was conducted during the summer on an intensively managed dairy farm in Canterbury, New Zealand (43° 35′ 30.6′′ S, 171° 55′ 36.6′′ E). The soil was a free-draining Lismore stony silt loam, known as a Pallic Firm Brown Soil in the New Zealand Soil Classification (Hewitt 2010b) or as a Xerepts Udepts Typic Dystrudepts in the USDA classification (Soil Survey Division Staff 1999), with a 150-mm-deep A (Ap) horizon consisting of fractions of 0.29, 0.12, and 0.58 of clay, sand, and slit, respectively (Carrick et al. 2013). The pasture consisted of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). A 6 × 6 m experimental area on the grazed paddock was fenced to exclude animals for 90 d before the start of the experiment and was shielded from irrigation and precipitation using a tunnel house covered with a transparent plastic cover (Torto). The paddock is normally mob-grazed every 3 to 4 wk throughout the growing season and is irrigated every 3 d when rainfall is insufficient.

4.3.2 Experimental Design

The experiment was a split-plot randomized block design with irrigation frequency as the main plot and urine addition or non-urine as the subplots. Each treatment combination was replicated four times (Supplemental Fig. S1). At the sampling locations, circular gas flux collars for gas sampling, supplementary collar bases for soil sampling, and instrumentation bases for marking the placement of automated sensors (area, 0.19635 m^2) were inserted into the soil to a depth of 100 mm. Irrigation frequency was either every 3 d (with 12 mm applied over a 10-min irrigation event, equivalent to 72 mm h^{-1}) or every 6 d (with 24 mm applied over a 10-min irrigation event, equivalent to 144 mm h^{-1}) and was applied over a ~0.2 m^{-2} area within each collar base. The 3-d treatment followed the current on-farm practice. The 6-d treatment reduced the frequency but increased the intensity. Irrigation was applied using an eight-branch manifold equipped with nozzles (Fulljet FL-5VG, Teejet Technologies) positioned 200 mm above the ground and controlled by an automated timer.

The day before urine treatment application is referred to herein as day of experiment (DOE) −1 (20 Feb. 2014). Urine was collected from the Lincoln University Dairy Farm on DOE −1 from cows fed ryegrass/white clover pastures, and 2 L of urine was applied to the soil within each urine -treated chamber base on DOE 0. The urine was applied once at a rate of 750 kg N ha^{-1}, which is typical of

cattle urine (Haynes and Williams 1993). The N content of the urine was determined by analyzing a subsample on a CN elemental analyzer (Vario-Max, Elementar GmbH). The non-urine subplots received neither urine nor water on this day to mimic actual field differences between soil affected and unaffected by urine patches.

4.3.3 Nitrous Oxide Fluxes

Soil-to-atmosphere N_2O fluxes were measured using vented insulated non–steady-state chambers (headspace volume, 19.625 L) following standardized protocols (Parkin et al. 2012). Fluxes were measured daily between 10:00 AM and 12:00 PM (van der Weerden et al. 2013) and were expressed as daily fluxes from DOE −1 and 29 and also on DOE 32 and 35. To seal chambers during sampling, annular moats on the bases were filled with water. Gas samples were taken at 0, 15, 30, and 45 min from each chamber using a 20-mL glass syringe fitted with a three-way stopcock and immediately transferred to 6-mL pre-evacuated (−1 atm) glass Exetainers (Labco Ltd.). Gas samples were analyzed on an automated gas chromatograph system equipped with an electron capture detector (SRI 8610c GC, SRI Instruments) as described in Clough et al. (1996). Flux calculations used the ideal gas law, air temperature, chamber volume and area, and the change in N_2O concentration over time, which was assessed using both quadratic regression (Wagner et al. 1997) and linear regression. The quadratic regression flux was selected unless the second derivative of the regression model was ≥0 (Venterea et al. 2009, Venterea 2013) according to the LINEST function in Microsoft Excel (version 2013). A correction factor was applied to account for chamber-induced artifacts using soil bulk density (Venterea 2010). Fluxes below the detection limit (Parkin et al. 2012) were assigned a value of zero. Of the 528 fluxes, 75% were calculated using the quadratic regression method, and 21% were calculated using the linear regression method. The remaining 4% were below the detection limit.

Cumulative N_2O emissions (kg N ha^{-1}) were determined by summing the daily fluxes. Emission factors (%) for N_2O lost as a proportion of urine-N were also determined (de Klein et al. 2003).

4.3.4 Ancillary Soil and Pasture Measurements

Sensors for soil O_2 (SO-110, Apogee Instruments), temperature (Probe 107, Campbell Scientific), and volumetric water content (θ_v) (CS 616 Reflectometer, Campbell Scientific) were installed in the center of the experimental plots inside the instrumentation collar bases (Supplemental Fig. S1). Soil O_2 and temperature sensors were installed at depths of 10, 50, and 100 mm, and the θ_v

sensors were installed at depths of 50 and 100 mm. A three-point linear calibration (0.5, 30, and 99% O_2 concentration) was used to calibrate the soil O_2 sensors. A change of 1% O_2 is equivalent to a 0.6-mV change in the sensor reading, and at an O_2 concentration of 20.95% (ambient), the measurements are repeatable at <0.1 mV (~0.2% O_2) (Apogee Instruments 2015). Each O_2 sensor was equipped with a diffusive head, which integrated an area of ~385 mm^2 around the sensor when placed in soil. Air temperature (Probe 107, Campbell Scientific) at 1.5 m above the soil surface and barometric pressure (SB-100, Apogee Instruments) at the soil surface were also measured. Two data loggers and a multiplexer powered and controlled the instrumentation (CR3000, CR1000, AM416, Campbell Scientific), with samples taken every 15 min from DOE −1 onward. Daily evapotranspiration (ET) was estimated from the Penman–Monteith equation (Allen et al. 1998) using wind speed ($m\ s^{-1}$), net radiation ($MJ\ m^{-2}\ d^{-1}$), and relative humidity (%) measured at a nearby meteorological station.

Bulk density was determined from within the chamber bases at the end of the experiment using the sand replacement method (Maynard and Curran 2008). Soil WFPS was calculated using the θ_v at soil depth of 50 mm (Linn and Doran 1984). Soil D_P/D_O was calculated using the structure-dependent, water-induced linear reduction model (Moldrup et al. 2013a), which uses air-filled pore space (Farquharson and Baldock 2008), total porosity, and a media complexity factor of 2.1 (Moldrup et al. 2013a).

The pasture was harvested to ~50 mm height on DOE 16 and 35. Dry matter (DM) yield ($kg\ ha^{-1}$) was determined after oven-drying for 48 h at 50°C.

Soil samples were collected on DOE −1, 5, 11, 17, 23, and 29 using a 70-mm-long auger from the supplementary bases allotted for soil collection for a total of four samples, which were not composited, from each treatment combination at each sampling time. Soils were extracted or analyzed within 24 h of collection and stored at 4°C until extraction or analysis. Gravimetric soil moisture (θ_g) was determined by oven-drying soil subsamples at 105°C for 24 h. Soil pH was determined with a pH probe (SevenEasy, Mettler Toledo) after mixing 10 g air-dried soil with 25 mL deionized water (Blakemore et al. 1987) after 12 h of settling. Nitrate (NO_3^-–N) and ammonium (NH_4^+–N) concentrations were determined by extracting 4 g dry weight equivalent soil with 40 mL 2 mol L^{-1} KCl. Samples were shaken for 1 h followed by 20 min of centrifuging at 2000 rpm before gravity filtering through Whatman no. 42 filters (Blakemore et al. 1987). Nitrite (NO_2^-–N) was extracted from 10 g dry weight equivalent soil using 40 mL 2 mol L^{-1} KCl adjusted to pH 8.0

(Stevens and Laughlin 1995). Extracts were shaken for 10 min and centrifuged for 5 min at 1500 rpm followed by gravity filtering through Whatman 42 filters (Stevens and Laughlin 1995). The NO_2^-–N extracts were analyzed within 24 h of extraction, and NO_3^-–N and NH_4^+–N extracts were frozen until flow injection analysis (FIAstar 5000 Analyzer, FOSS Analytical).

Cold water–extractable carbon (CWC) was measured using 3 g of soil and 30 mL of deionized H_2O shaken for 30 min and centrifuged at 3500 rpm followed by filtering through Avantec 5C filters (Ghani et al. 2003). After filtration, soil was extracted a second time for hot water carbon (HWC), as described by Ghani et al. (2003). The CWC and HWC extracts were frozen until analysis on a total organic carbon analyzer (TOC 5000A, Shimadzu).

Potential denitrification enzyme activity (DEA) was determined using the acetylene (C_2H_2) block technique (Groffman et al. 2006, Drury et al. 2008). Briefly, 25 mL of a solution containing 50 μg g^{-1} of NO_3^-–N (as KNO_3) and 300 μg g^{-1} of C (as HWC extracted from the same soil used for the denitrification potential measurement) was mixed with 20 g dry weight equivalent of soil and placed in a 250-mL Mason jar with a gas-tight lid fitted with a rubber septum. The jar headspace was made anaerobic by flushing the jar with N_2 (instrument grade, <0.0001% O_2) for 10 min and then incubating with acetylene (+C_2H_2, instrumentation grade C_2H_2 >98%, <2% air) or without acetylene (–C_2H_2) at 20°C for 48 h. The headspace of the jars was sampled using a closed-loop circulating system attached to the photo-acoustic analyzer (multi-gas monitor type 1302, Brüel and Kjaer) to measure N_2O. The jars and the closed-loop system were flushed with N_2 gas; exhaust was directed into a container of water to keep pressure equilibrated within the closed loop and the jar and to minimize O_2 leakage back into the system. During sampling for N_2O, the inlet for the N_2 and the outlet to the water were closed. The change in N_2O concentration was measured every 2 min for 8 min. Each jar was measured every 4 h for the first 24 h and every 8 h thereafter. Total N_2O evolved over each 48-h incubation period represented either DEA-N_2O + N_2 (from the +C_2H_2 samples) or DEA-N_2O (from the –C_2H_2 samples), which were then expressed as the $N_2O/(N_2O + N_2)$ ratio; herein this ratio is referred to as DEA-N_2O/(DEA-N_2O + N_2).

4.3.5 Data Analyses

All analyses were performed in Minitab (Minitab Inc. version 17 2010) unless otherwise specified. Data were transformed (Supplemental Table S1) to meet assumptions of parametric statistics when required (Steel et al. 1997). Statistical analyses for treatment effects did not include data prior to urine application (DOE –1 and 0), but these data are presented for reference. When data

were transformed, conclusions were drawn from the analysis on the transformed scale; however, the mean and error values presented in tables and figures are from untransformed data.

Treatment effects on mean daily N_2O emissions were evaluated using a linear mixed model in SPSS (I. B. M. Corp 2011). Irrigation frequency, urine, and DOE were treated as fixed effects, with DOE as a repeated measure using a heterogeneous first-order autoregressive covariance structure. *P* values of $\leq$0.10 are considered significant. For NH_4^+–N, NO_3^-–N, NO_2^-–N, HWC, CWC, soil pH, and θ_g, a general linear model was used to evaluate treatment effects. Volumetric water content data could not be transformed to normal because the distribution was bimodal, so these data were not analyzed statistically for treatment effects. Irrigation frequency, urine, DOE, and interactions were treated as fixed factors. Main effects were tested using Tukey's multiple comparison test (Steel et al. 1997).

A general linear model was used to test for treatment effects with irrigation frequency and urine as factors and with interaction effects assessed between urine $\times$ irrigation frequency for cumulative N_2O emissions acquired individually from each chamber; DM yield; pasture N content; daily averaged soil temperature at 50 mm; daily average soil O_2 at 10, 50, and 100 mm; and the ratio of DEA-N_2O/DEA-N_2O + N_2.

Least squares linear regression was used to evaluate relationships with daily N_2O fluxes, cumulative N_2O fluxes, or DEA-N_2O/(DEA-N_2O + N_2) as the response variables and with NH_4^+–N; NO_3^-–N; NO_2^-–N; HWC; CWC; soil pH; θ_g; daily average soil temperature at 50 mm; daily average soil O_2 at 10, 50, and 100 mm; daily average WFPS; and daily average D_P/D_O as the explanatory variables.

4.4 Results

4.4.1 Soil Physical Properties

Spikes in θ_v were observed after irrigation events and after the urine deposition event (Figure 4.1 c, d). Overall mean θ_g (Figure 4.1 a,b) was 7% higher under the 3-d irrigation treatment than under the 6-d irrigation treatment ($P < 0.001$) and 17% higher in the urine-treated compared with the non–urine-treated soil ($P < 0.001$). Total irrigation exceeded total evapotranspiration in the non-urine and urine treatments by 41.0 and 52.4 mm, respectively.

Overall mean soil temperatures at 50 mm from the urine, non-urine, 3-d, and 6-d irrigation treatments were 15.4 ± 022, 15.7 ± 0.19, 16.1 ± 0.22, and 15.0°C ± 0.18, respectively. Overall mean soil temperatures were higher under the 3-d irrigation treatment than under the 6-d irrigation treatment ($P < 0.05$). The addition of urine did not influence soil temperature (Supplemental Fig. S2).

Soil O_2 showed diel variation (Supplemental Fig. S3). After the urine application, soil O_2 decreased to a minimum of 13% at 100 mm soil depth and recovered to pretreatment concentrations within 24 h. Between DOE 1 and 35 (the data used for statistical analysis), daily mean soil O_2 concentrations varied between 17 and 20% (Figure 4.1 e-h). Overall mean soil O_2 concentrations in the 3-d irrigation treatment were 1.09 and 0.79% lower at 50 ($P < 0.001$) and 100 mm ($P < 0.001$) soil depths, respectively, when compared with the 6-d irrigation treatment. The overall average soil O_2 concentration at 10 mm was 0.32% lower in the urine treatment compared with the non-urine treatment ($P < 0.01$). Lower soil O_2 was found with both urine and 3-d irrigation treatment at 50 and 100 cm ($P < 0.05$).

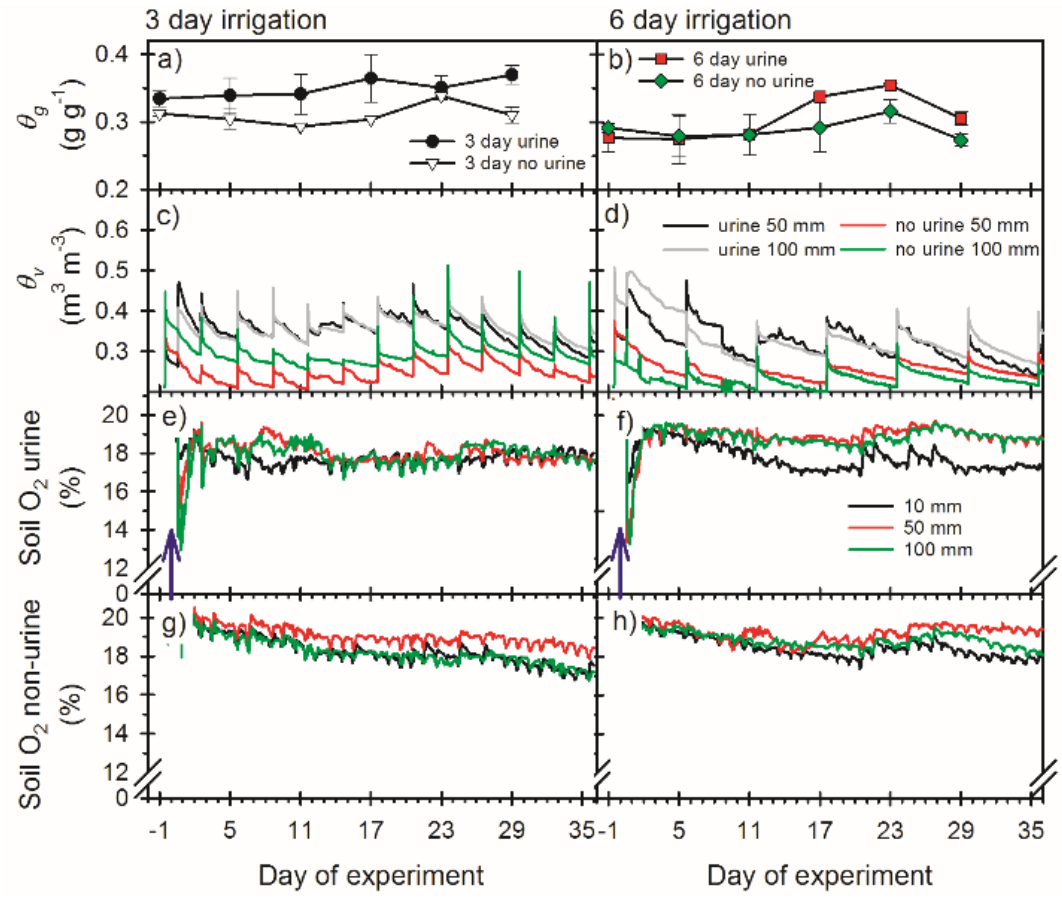


Figure 4.1 Average gravimetric soil moisture (ϑ_g), volumetric water content (ϑ_v), soil oxygen (O_2) from the urine- and non-urine-treatment from the 3-d (a, c, e, and g), and 6-d irrigation treatment (b, d, f, and h). The arrow represents the timing of urine deposition.

Relative soil gas diffusivity, D_P/D_O, ranged from 0.026 to 0.101, averaging 0.050, 0.029, 0.089, and 0.031 in the 3-d non-urine, 3-d urine, 6-d non-urine, and 6-d urine treatments, respectively. The WFPS ranged from 0.24 to 0.45 $m^3\ m^{-3}$, averaging 0.26, 0.41, 0.29, and 0.34 $m^3\ m^{-3}$ from the 3-d non-urine, 3-d urine, 6-d non-urine, and 6-d urine treatments, respectively. Urine increased overall mean WFPS ($P < 0.001$) and decreased D_P/D_O ($P < 0.001$). Under the 6-d irrigation treatment, WFPS was lower ($P < 0.001$) and D_P/D_O was higher ($P < 0.001$) compared with the 3-d irrigation treatment. There was an interaction between urine and irrigation treatments, with D_P/D_O being lower under the 3-d irrigation treatment with urine application ($P < 0.001$).

4.4.2 Soil Chemical Properties

Urine application increased overall mean concentrations of NO_3^-–N (Figure 4.2 d) and NH_4^+–N (Figure 4.2 b) and increased soil pH ($P < 0.05$) (Figure 4.2 h), with NH_4^+–N peaking shortly after urine deposition (Figure 4.2 a) and NO_3^-–N increasing with time since urine deposition (Figure 4.2

b). The addition of urine did not affect the HWC values (Figure 4.2 k), but the 6-d irrigation frequency resulted in 20% higher HWC ($P < 0.05$) (Figure 4.2 l). Urine and irrigation treatments interacted to produce greater soil NO_3^-–N and NH_4^+–N concentrations under urine in the 6-d irrigation treatment ($P < 0.10$). Concentrations of NO_3^-–N (Figure 4.2 e) and CWC (Figure 4.2 i) differed with DOE but were not influenced by urine or irrigation treatments (Figure 4.2 f-j).

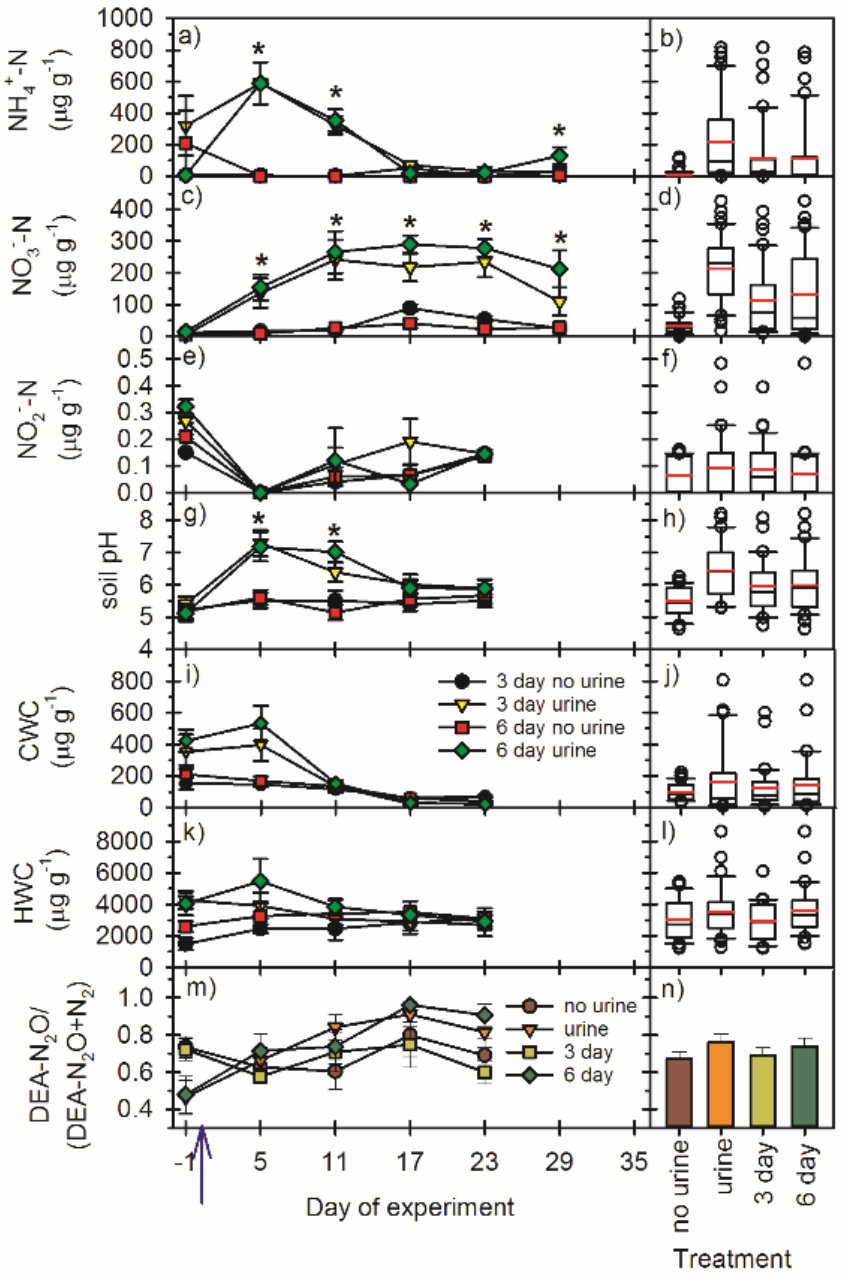


Figure 4.2 Changes to means over time (±SEM, n = 4) for each treatment combination and box plots for each irrigation frequency and urine treatment (±SEM, n = 32), for NH_4^+-N (a, b), NO_3^--N (c, d), NO_2^--N (e, f), soil pH (g, h), cold-water carbon (CWC, i, j), hot-water carbon (HWC, k, l), and ratio of N_2O/N_2O+N_2 from denitrification enzyme assays (DEA, ±SEM, n = 6), and overall mean (± SEM, n = 6) of the ratios of N_2O/N_2O+N_2 from each treatment (m, n). The blue arrow represents the timing of urine deposition. The differences at $P < 0.05$ between urine and non-urine treatments are represented by an asterisk. The box plots represent the data by treatment as analyzed statistically. In the box plots, the median is represented by the grey line and the mean is represented by the red line. The box represents the 25th and 75th percentile, the whiskers represent the smallest and largest values that are not considered outliers, and the circles represent outliers.

4.4.3 Pasture Yield

Irrigation frequency did not influence DM yield. Urine application increased total DM yield by 35% ($P < 0.05$) over the whole experimental period from 2634.7 kg ha^{-1} (SEM, 227.0) to 3754.0 kg ha^{-1} (SEM, 146.2). Dry matter yields were 19% higher from the urine treatment compared with the non-urine treatment at the first harvest ($P < 0.10$) and were 47% higher from the second cut ($P < 0.05$).

4.4.4 Nitrous Oxide Fluxes

The daily N_2O fluxes from the urine treatment varied with DOE ($P < 0.001$) (Figure 4.3 a). Overall mean daily N_2O fluxes from the urine treatment were 440% higher compared with the non-urine treatment ($P < 0.001$) (Figure 4.3 b). Non-urine N_2O fluxes were low (≤1.2 mg N m^{-2} d^{-1}), with an overall average of 0.47 mg N m^{-2} d^{-1}. Daily N_2O fluxes did not differ with irrigation treatment.

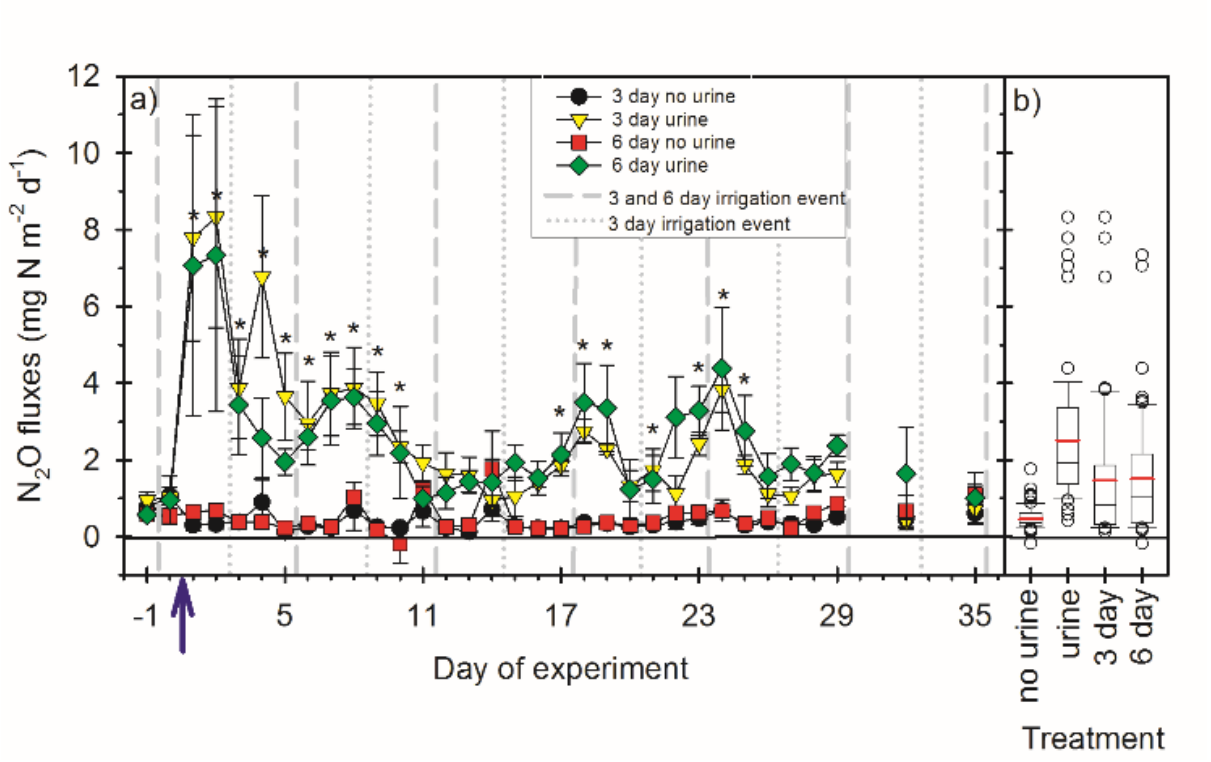


Figure 4.3 a) Mean daily N_2O fluxes from each treatment (±SEM, n = 4) where the arrow represents the timing of urine deposition. Differences between the urine- and non-urine-treatments ($P < 0.05$) on each day are represented by an asterisk. b) A box plot comparison of daily N_2O emission from each treatment as analyzed statistically (±SEM, n = 256). In the box plots, the grey line represents the median and the red line represents the mean. The box represents the 25th and 75th, and the open circles represent outliers.

The cumulative N_2O emissions (data not shown) reflected the trends observed in the daily N_2O fluxes and were higher under urine by a factor of 4.9 ($P < 0.001$) compared with the non-urine treatment. Irrigation frequency did not influence cumulative N_2O emissions. When expressed as an emission factor, cumulative N_2O emissions from the 3-d and 6-d irrigation treatments equaled 0.09%.

Nitrous oxide fluxes were highest between 0.4 and 0.6 $m^3\ m^{-3}$ WFPS (Figure 4.4 a) and were highest from D_P/D_O values between ≈0.06 and ≈0.02 (Figure 4.4 b). Pooling all N_2O flux data, irrespective of treatment, and performing linear regression analysis of log-transformed WFPS or D_P/D_O versus log-transformed daily N_2O fluxes showed that D_P/D_O best explained the variation in the daily N_2O fluxes (Figure 4.4 c, d). Overall mean WFPS and D_P/D_O explained 16% (not significant) and 87% ($P < 0.05$) of the variability in cumulative N_2O emissions from urine-treated soils, respectively (Figure 4.4 e, f).

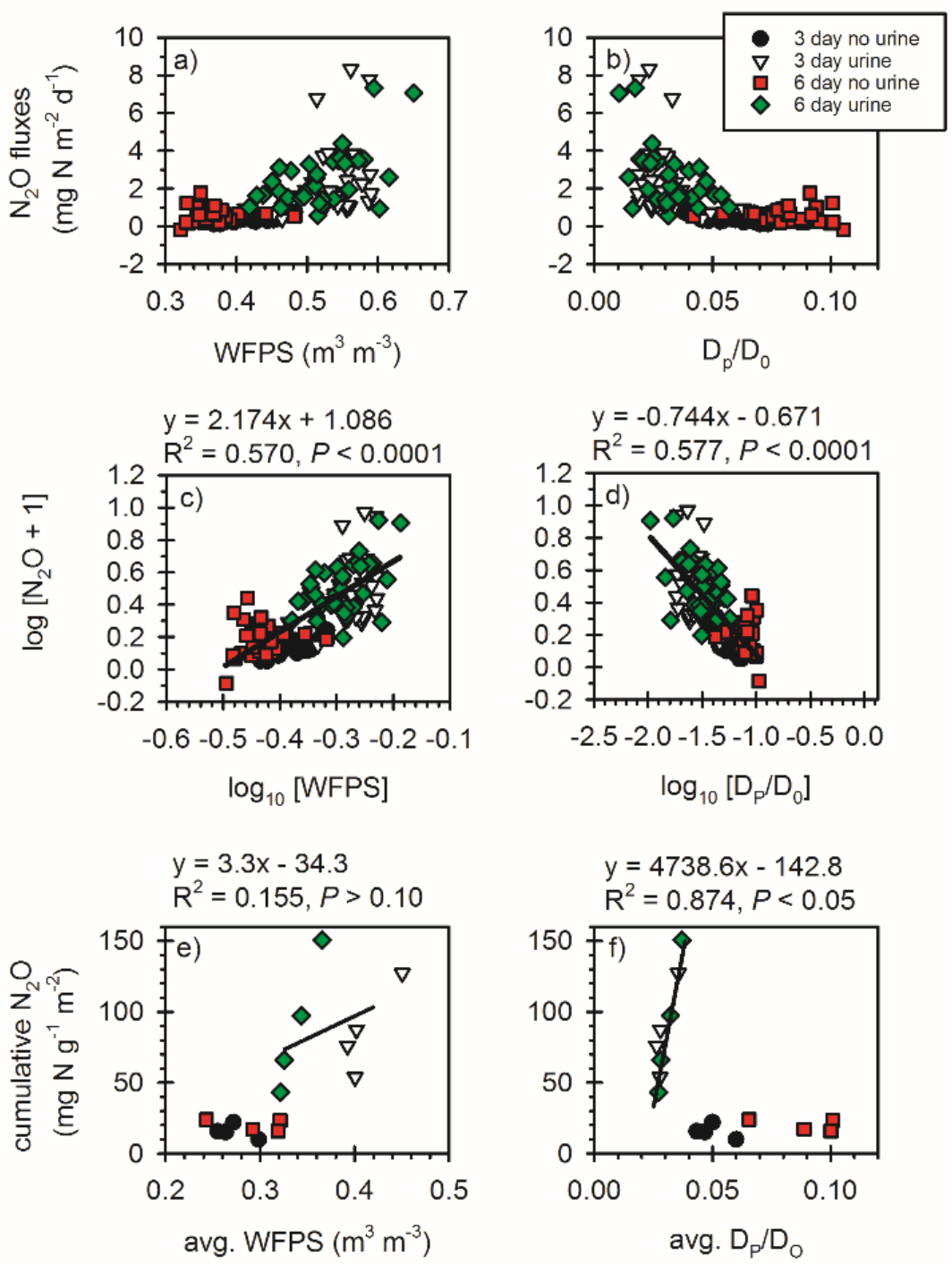


Figure 4.4 The daily average nitrous oxide (N_2O) fluxes and a) water-filled pore space (WFPS) and b) relative soil diffusivity (D_P/D_O). Linear regression between average log_{10} [1+ N_2O] and c) log_{10} [WFPS] or d) log_{10} [D_P/D_O] from data from all treatments. The linear regression between cumulative N_2O fluxes from the urine-treatment and e) overall mean WFPS from the urine-treatment or f) overall mean D_P/D_O from the urine-treatment.

Concentrations of NO_3^-–N and NH_4^+–N and soil pH explained 18 ($P < 0.05$), 28 ($P < 0.001$), and 32% ($P < 0.001$) of the variability in daily N_2O fluxes, respectively, under the 3-d irrigation frequency. However, there were no relationships observed between daily N_2O fluxes and environmental variables under the 6-d irrigation treatment. When all of the data were pooled, irrespective of treatment, NO_3^-–N, NH_4^+–N, NO_2^-–N, and pH explained 10 ($P < 0.05$), 18 ($P < 0.001$), 12 ($P < 0.05$), and 13% ($P < 0.05$) of the variability in daily N_2O fluxes, respectively.

4.4.5 Ratios of DEA-N_2O/(DEA-N_2O+N_2) from Denitrification Enzyme Assays

The overall mean ratio of DEA-N_2O/(DEA-N_2O + N_2) was greater from the 6-d (0.83) compared with the 3-d (0.65) irrigation treatment ($P < 0.05$) and was lower from the non-urine (0.67) compared with the urine (0.81) treatments ($P < 0.05$) (Figure 4.2 n). There was an interaction between the treatments, with a lower ratio observed from the 3-d and non-urine treatment ($P < 0.05$). These treatment differences were also reflected in the temporal trends. By DOE 17 and 23, the ratios of DEA-N_2O/(DEA-N_2O + N_2) were 0.98 and 0.95, respectively, under the 6-d irrigation treatment and 0.81 and 0.60, respectively, under the 3-d irrigation treatment (Figure 4.2 m). The ratio of DEA-N_2O/(DEA-N_2O + N_2) was positively related to CWC ($R^2 = 0.23$; $P < 0.10$) and negatively related to NO_3^-–N ($R^2 = 0.28$; $P < 0.05$).

4.5 Discussion

Other studies have reported similar N_2O emissions from free-draining soil both for the peak urine-induced (Di and Cameron 2002b) and the average non-urine emissions (Horváth et al. 2010). Cumulative N_2O emissions (Di and Cameron 2002b) and emission factors (de Klein et al. 2014) are within the range of those reported by others from free-draining soil that received cow urine of similar concentrations. Urine application results in a series of hydrolysis reactions, followed by biological nitrification and denitrification (Baral et al. 2014), which subsequently change the soil pH and inorganic N concentrations (Orwin et al. 2010, Taghizadeh-Toosi et al. 2011). Although these factors are known regulators of N_2O fluxes (Firestone and Davidson 1989), individually they were not robust predictors of N_2O fluxes in this study. Rather, they contributed to the variability in N_2O fluxes observed between urine treatments. The lack of any irrigation frequency effects on N_2O emissions can be explained by considering how N_2O regulators varied, specifically soil O_2 concentration and D_P/D_O. As originally hypothesized, more frequent irrigation produced higher soil moisture and lower soil O_2, and the DEA-N_2O/(DEA-N_2O+N_2) ratio was lower, inferring greater

potential for N_2OR activity and thus a greater reduction of N_2O to N_2. However, this did not result in lower N_2O emissions.

The higher overall mean soil θ_g under the urine treatment could have resulted from the additional water embodied in the applied urine, equal to 10.8 mm irrigation or 7.5% more total water. Despite equal volumes of water being applied in total, the soil was drier under the 6-d irrigation treatment most of the time. Higher irrigation intensity can increase preferential flow through macropores as a consequence of an increasing hydrostatic head due to the increased water application rates (Gjettermann et al. 1997). The relatively drier soil conditions under the 6-d irrigation treatment suggest this occurred.

Although N_2O fluxes were not affected by irrigation, daily average N_2O fluxes did increase with increasing WFPS and declining D_P/D_O (Figure 4.4 a, b). Soil D_P/D_O is a measure of the relative rate at which O_2 diffuses through soil and takes into account pore water blockage effects. Oxygen diffuses about 10^4 times slower in water than in free air, and thus soil moisture content exerts a major influence on soil D_P/D_O (Moldrup et al. 2001, Farquharson and Baldock 2008, Moldrup et al. 2013a). Soil WFPS is often used to explain N_2O flux magnitude (Velthof and Oenema 1995, Smith et al. 1998, Dobbie et al. 1999), but the relationship does not account for the interaction between bulk density and matric potential (Balaine et al. 2013). Soil D_P/D_O does account for these variations, and this explains the strong relationship observed between N_2O fluxes and D_P/D_O (Figure 4.4 d, f). In this study, log-transformed daily average N_2O fluxes related well to both log-transformed WFPS and log-transformed D_P/D_O under the controlled range of soil moisture. However, the inclusion of physical differences in the soil using D_P/D_O provides a repeatable threshold for N_2O production and consumption (Balaine et al. 2013, Harrison-Kirk et al. 2015).

Soil anaerobiosis has been reported to begin at D_P/D_O <0.02 (Stepniewski 1981), suggesting the soils were well aerated during the current experiment (Figure 4.4 b, f). This is supported by the fact that soil O_2 concentrations did not fall below 17% except immediately after the urine application. Higher soil water content under the 3-d irrigation treatment impeded soil O_2 replenishment via diffusion from the atmosphere to the soil. This, combined with the low variability in daily mean soil O_2 concentrations, explains the lower soil O_2 observed at 50 and 100 mm in the 3-d irrigation treatment.

The diel variation in soil O_2, which lagged soil temperature, was most likely driven by heterotrophic soil respiration (Lloyd and Taylor 1994). Despite the soil being well aerated (D_P/D_O value >0.02 and

soil O_2 >17%), daily N_2O fluxes from the urine treatments after DOE 17 imply N_2O emissions occurred via denitrification because NO_3^-–N was the only available substrate. Denitrification or nitrifier–denitrification in anaerobic microsites must have contributed to N_2O emissions under otherwise aerated soil conditions (Müller et al. 2004, Morley et al. 2008). Thus, measured O_2 concentrations during this study did not reflect soil O_2 concentrations at microsites, and a method to measure soil O_2 in situ at the microscale is still required.

Urine addition decreased soil O_2 for ~24 h. This is consistent with urea hydrolysis reactions that occur after urine deposition, which take between 24 and 48 h (Sherlock and Goh 1983). The hydrolysis reactions create OH^- ions, increase pH, and generate NH_4^+ and bicarbonate ions, with the latter hydrolyzing to generate CO_2 (Avnimelech and Laher 1977). Fluxes of CO_2 have been previously observed immediately after urine deposition (Uchida et al. 2008). Rapid anoxia from CO_2 production may trigger denitrification (Sherlock and Goh 1983), accounting for high N_2O fluxes after urine deposition.

Nitrous oxide production and N_2OR activity via heterotrophic denitrification and nitrifier–denitrification pathways occur under anaerobic or anoxic conditions, respectively (Wrage et al. 2001, Zhu et al. 2013). The strong relationship between net N_2O emissions and average D_P/D_O suggests D_P/D_O (Figure 4.4 f) could provide insight into the potential for N_2O uptake. The DEA-N_2O/(DEA-N_2O + N_2) ratios were positively related to C, which is a driver of denitrification (Barnard et al. 2005) and negatively related to NO_3^-–N, which is preferentially used over N_2O as a terminal electron acceptor during denitrification (Barnard et al. 2005). The denitrification enzyme assays were run under nonlimiting conditions and therefore do not directly reflect *in situ* conditions. These assay results demonstrate a proof-of-concept; even when bulk soil O_2 is not anaerobic, the contribution of anaerobic microsites can have a significant impact on the ratio of $N_2O/(N_2O + N_2)$ emitted. Future research using ^{15}N isotopes for partitioning N_2O/N_2 ratios, along with direct measurements of N_2OR, are required and should be linked to D_P/D_O to refine its use for predicting O_2–N_2O relationships in grazed pasture soils.

From the perspectives of farm and water management, this study shows that, on a free-draining soil, increasing the irrigation frequency while providing the same total volume of water does not enhance N_2O emissions or alter DM production rates within ruminant urine patches. There may be the potential for higher N_2 losses as irrigation intensity increases, but this needs to be confirmed with further study.

4.6 Conclusions

Daily and cumulative N_2O emissions and DM yields were not influenced by irrigation frequency. A lower ratio of DEA-N_2O/(DEA-N_2O + N_2) indicated greater potential for N_2OR activity and therefore greater potential for N_2O to be reduced to N_2 in the more frequently irrigated treatment, but this was not reflected in the field N_2O emissions. Estimates of D_P/D_O are a good indicator of cumulative N_2O emissions in urine-treated soils and explain well the variability in daily N_2O emissions. Future work linking D_P/D_O and soil O_2 is needed in other soil types and under different climatic and moisture conditions to improve our understanding of the effects of irrigation frequency on N_2O emissions and $N_2O/(N_2O + N_2)$ ratios.

Acknowledgements

This research was undertaken with CRI Core Funding to Landcare Research from New Zealand's Ministry of Business, Innovation and Employment. We are grateful for the help in the field provided by Neil Smith and Greg Curlin, and to the Centre for Environment and Soil Research at Lincoln University for the loaned equipment. Synlait Farms (now renamed Purata) kindly allowed access to one of their dairy farms.

Supplementary material

The supplementary data includes more information on data transformations for statistics, a map of the experimental plot, soil temperature time series, and an example of the diel cycling of soil O_2 and soil temperature.

4.7 References

Allen, R., L. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration - Guidelines for computing crop water requirements - FAO Irrigation and drainage paper 56.

Apogee Instruments, I. 2015. Apogee Instruments Owner's Manual Oxygen Sensor Models SO-110, SO-120, SO-210, and SO-220.

Avnimelech, Y., and M. Laher. 1977. Ammonia volatilization from soils:Equilibrium considerations. Soil Science Society of America Journal 41:1080-1084.

Balaine, N., T. Clough, M. Beare, S. Thomas, E. Meenken, and J. Ross. 2013. Changes in Relative Gas Diffusivity Explain Soil Nitrous Oxide Flux Dynamics. Soil Science Society of America Journal 77:1496-1505.

Baral, K., A. Thomsen, J. Olesen, and S. Petersen. 2014. Controls of nitrous oxide emission after simulated cattle urine deposition. Agriculture, Ecosystems and Environment 188:103-110.

Barnard, R., P. Leadley, and B. Hungate. 2005. Global change, nitrification, and denitrification: A review. Global Biogeochemical Cycles 19:GB1007.

Blakemore, L. C., P. L. Searle, and B. K. Daly. 1987. Methods for chemical analysis of soils. Pages 7-29 NZ Soil Bureau Scientific Report.

Carrick, S., T. Webb, J. Scott, and J. Payne. 2013. Stony soil physical attribute characterisation report - Progress for 2012/13 years. Unpublished Internal Report, Lincoln, New Zealand.

Clough, T. J., R. R. Sherlock, K. C. Cameron, and S. F. Ledgard. 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant and Soil 178:141-152.

Davidson, E. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nature Geoscience 2:659-662.

de Klein, C., L. Barton, R. Sherlock, Z. Li, and R. Littlejohn. 2003. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. Australian Journal of Soil Research 41:381-399.

de Klein, C., J. Luo, B. Woodward, T. Styles, B. Wise, S. Lindsey, and N. Cox. 2014. The effect of nitrogen concentration in synthetic cattle urine on nitrous oxide emissions. Agriculture, Ecosystems and Environment 188:85-92.

Di, H. J., and K. C. Cameron. 2002. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use and Management 18:395-403.

Dobbie, K. E., I. P. McTaggart, and K. A. Smith. 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research 104:26891-26899.

Drury, C. F., D. D. Myrold, E. G. Beauchamp, and W. D. Reynolds. 2008. Denitrificaiton techniques for soil. Pages 471-493 in M. R. Carter and E. G. Gregorich, editors. Soil Sampling and Methods of Analysis. CRC Press, Boca Raton, FL, USA.

Eccles, W. J., C. Matthew, and A. C. P. Chu. 1990. Response of Matua prairie grass and Ellett perennial ryegrass to excess soil moisture in sand, silt and clay soils. Proceedings of the New Zealand Grassland Association 51:127-130.

Farquharson, R., and J. Baldock. 2008. Concepts in modelling N_2O emissions from land use. Plant and Soil 309:147-167.

Firestone, M., and E. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil.in M. O. Andreae and D. S. Schimel, editors. Exchange of Trace Gases Between Ecosystems and the Atmosphere. Wiley, New York.

Food, and N. Agriculture Organization of the United. 2010. Statistical Yearbook.

Ghani, A., M. Dexter, and K. W. Perrott. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology and Biochemistry 35:1231-1243.

Gjettermann, B., K. L. Nielsen, C. T. Petersen, H. E. Jensen, and S. Hansen. 1997. Preferential flow in sandy loam soils as affected by irrigation intensity. Soil Technology 11:139-152.

Groffman, P., M. Altabet, J. K. Böhlke, K. Butterbach-Bahl, M. David, M. Firestone, A. Giblin, T. Kana, L. P. Nielsen, and M. Voytek. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. Ecological Applications 16:2091-2122.

Harrison-Kirk, T., S. Thomas, T. Clough, M. Beare, T. van der Weerden, and E. Meenken. 2015. Compaction influences N_2O and N_2 emissions from ^{15}N-labeled synthetic urine in wet soils during successive saturation/drainage cycles. Soil Biology and Biochemistry 88:178-188.

Haynes, R. J., and P. H. Williams. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49:119-199.

Hewitt, A. E. 2010. New Zealand Soil Classification. Manaaki-Whenua Press.

Horváth, L., B. Grosz, A. Machon, Z. Tuba, Z. Nagy, S. Z. Czóbel, J. Balogh, E. Péli, S. Z. Fóti, and T. Weidinger. 2010. Estimation of nitrous oxide emission from Hungarian semi-arid sandy and loess grasslands; effect of soil parameters, grazing, irrigation and use of fertilizer. Agriculture, Ecosystems and Environment 139:255-263.

I. B. M. Corp. 2011. IBM SPSS Statistics for Windows Version 20.0. Armonk, NY:IBM Corp.

IPCC. 2007. Intergovernmental Panel on Climate Change, Climate Change: Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Linn, D. M., and J. W. Doran. 1984. Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. Soil Science Society of America Journal 48:1267-1272.

Lloyd, J., and J. A. Taylor. 1994. On the Temperature Dependence of Soil Respiration. Functional Ecology 8:315-323.

Maharjan, B., R. Venterea, and C. Rosen. 2014. Fertilizer and Irrigation Management Effects on Nitrous Oxide Emissions and Nitrate Leaching. Agronomy Journal 106:703-714.

Maynard, D. G., and M. P. Curran. 2008. Bulk Density Measurement in Forest Soils. Pages 863-869 in M. R. Carter and E. G. Gregorich, editors. Soil Sampling and Methods of Analysis. Taylor & Francis group.

McBride, S. D. 1994. Pasture yield responses to irrigation in Canterbury. Proceedings of the New Zealand Grassland Association 56:165-168.

Minitab Inc. version 17. 2010. Minitab Statistical Software [Computer software]. State College, PA.

Moir, J. L., K. C. Cameron, H. J. Di, and U. Fertsak. 2011. The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. The Journal of Agricultural Science 149:473-485.

Moldrup, P., T. K. K. Chamindu Deepagoda, S. Hamamoto, T. Komatsu, K. Kawamoto, D. E. Rolston, and L. Wollesen de Jonge. 2013. Structure-Dependent Water-Induced Linear Reduction Model for Predicting Gas Diffusivity and Tortuosity in Repacked and Intact Soil. Vadose Zone Journal 12:2-11.

Moldrup, P., T. Olesen, T. Komatsu, P. Schjønning, and D. E. Rolston. 2001. Tortuosity, Diffusivity, and Permeability in the Soil Liquid and Gaseous Phase. Soil Science Society of America Journal 65:613-623.

Morley, N., E. Baggs, P. Dörsch, and L. Bakken. 2008. Production of NO, N_2O and N_2 by extracted soil bacteria, regulation by NO_2^- and O_2 concentrations. FEMS Microbiology Ecology 65:102-112.

Müller, C., R. J. Stevens, R. J. Laughlin, and H. J. Jäger. 2004. Microbial processes and the site of N_2O production in a temperate grassland soil. Soil Biology and Biochemistry 36:453-461.

Oenema, O., N. Wrage, G. Velthof, J. van Groenigen, J. Dolfing, and P. Kuikman. 2005. Trends in Global Nitrous Oxide Emissions from Animal Production Systems. Nutrient Cycling in Agroecosystems 72:51-65.

Orwin, K. H., J. E. Bertram, T. J. Clough, L. M. Condron, R. R. Sherlock, M. O'Callaghan, J. Ray, and D. B. Baird. 2010. Impact of bovine urine deposition on soil microbial activity, biomass, and community structure. Applied Soil Ecology 44:89-100.

Parkin, T. B., R. T. Venterea, and S. K. Hargreaves. 2012. Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. Journal of Environmental Quality 41:705-715.

Petersen, S., P. Ambus, L. Elsgaard, P. Schjønning, and J. Olesen. 2013. Long-term effects of cropping system on N_2O emission potential. Soil Biology and Biochemistry 57:706-712.

Ravishankara, A. R., J. Daniel, and R. Portmann. 2009. Nitrous Oxide (N_2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. Science 326:123-125.

Ruser, R., H. Flessa, R. Russow, G. Schmidt, F. Buegger, and J. C. Munch. 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. Soil Biology and Biochemistry 38:263-274.

Scheer, C., P. R. Grace, D. W. Rowlings, and J. Payero. 2013. Soil N_2O and CO_2 emissions from cotton in Australia under varying irrigation management. Nutrient Cycling in Agroecosystems 95:43-56.

Sherlock, R. R., and K. M. Goh. 1983. Initial emission of nitrous oxide from sheep urine applied to pasture soil. Soil Biology and Biochemistry 15:615-617.

Simojoki, A., and A. Jaakkola. 2000. Effect of nitrogen fertilization, cropping and irrigation on soil air composition and nitrous oxide emission in a loamy clay. European Journal of Soil Science 51:413-424.

Smith, K. A., P. E. Thomson, H. Clayton, I. P. McTaggart, and F. Conen. 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmospheric Environment 32:3301-3309.

Soil Survey Division Staff. 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. U. S. Department of Agriculture.

Steel, G. C., J. H. Torrie, and D. A. Dikey. 1997. Principles and procedures of statistics: A biometrical approach. McGraw-Hill.

Stepniewski, W. 1981. Oxygen diffusion and the strength as related to soil compaction. II Oxygen diffusion coefficient. Polish Journal of Soil Science 14:3-13.

Stevens, R. J., and R. J. Laughlin. 1995. Nitrite Transformations during Soil Extraction with Potassium Chloride. Soil Science Society of America Journal 49:933-938.

Taghizadeh-Toosi, A., T. Clough, L. Condron, R. Sherlock, C. Anderson, and R. Craigie. 2011. Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from ruminant urine patches. Journal of Environmental Quality 40:468-476.

Trost, B., A. Prochnow, K. Drastig, A. Meyer-Aurich, F. Ellmer, and M. Baumecker. 2013. Irrigation, soil organic carbon and N_2O emissions. A review. Agronomy for Sustainable Development 33:733-749.

Uchida, Y., T. Clough, F. Kelliher, and R. Sherlock. 2008. Effects of aggregate size, soil compaction, and bovine urine on N_2O emissions from a pasture soil. Soil Biology and Biochemistry 40:924-931.

van der Weerden, T. J., T. J. Clough, and T. M. Styles. 2013. Using near-continuous measurements of N_2O emission from urine-affected soil to guide manual gas sampling regimes. New Zealand Journal of Agricultural Research 56:60-76.

Velthof, G. L., and O. Oenema. 1995. Nitrous oxide fluxes from grassland in the Netherlands: II. Effects of soil type, nitrogen fertilizer application and grazing. European Journal of Soil Science 46:541-549.

Venterea, R. 2010. Simplified method for quantifying theoretical underestimation of chamber-based trace gas fluxes. Journal of Environmental Quality 39:126-135.

Venterea, R. 2013. Theoretical Comparison of Advanced Methods for Calculating Nitrous Oxide Fluxes using Non-steady State Chambers. Soil Science Society of America Journal 77:709-720.

Venterea, R., K. Spokas, and J. Baker. 2009. Accuracy and Precision Analysis of Chamber-Based Nitrous Oxide Gas Flux Estimates. Soil Science Society of America Journal 73:1087-1093.

Wagner, S. W., D. C. Reicosky, and R. S. Alessi. 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal 89:279-284.

Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry 33:1723-1732.

Zhu, X., M. Burger, T. Doane, and W. Horwath. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proceedings of the National Academy of Sciences 110:6328-6333.

4.8 Supplementary Data

Supplemental Table S1 Data transformations for the statistical analysis for experimental data between DOE 1 and DOE 35, where N/A represents no transformation required.

	Variable (units)	Transform
N_2O fluxes	Daily N_2O emissions (mg N g^{-1} m^{-2})	Ln (value +1)
	Cumulative N_2O emissions (mg N g^{-1} m^{-2})	ʎ = -0.50
soil chemical data	Nitrate (µg g^{-1} dry soil)	(value +1), ʎ = 0.5
	Ammonium (µg g^{-1} dry soil)	(value +1), Ln
	Nitrite (µg g^{-1} dry soil)	N/A
	Hot water carbon (µg g^{-1} dry soil)	ʎ = -3
	Cold water carbon (µg g^{-1} dry soil)	Ln
	Soil pH	ʎ = -3
environmental data	Gravimetric soil moisture (g g^{-1})	N/A
	Soil temperature 10 mm (°C)	Ln
	Soil temperature 50 mm (°C)	Ln
	Soil temperature 100 mm (°C)	ʎ = 0.5
	Soil oxygen 10 mm (°C)	ʎ = -5
	Soil oxygen 50 mm (°C)	N/A
	Soil oxygen 100 mm (°C)	N/A
soil physical data	Water-filled pore space (m^3 m^{-3})	ʎ = -0.5
	Soil diffusivity (D_P/D_O)	ʎ = 1
denitrification enzyme assay	Ratio DEA-N_2O/(DEA-N_2O+N_2)	ʎ = -0.5

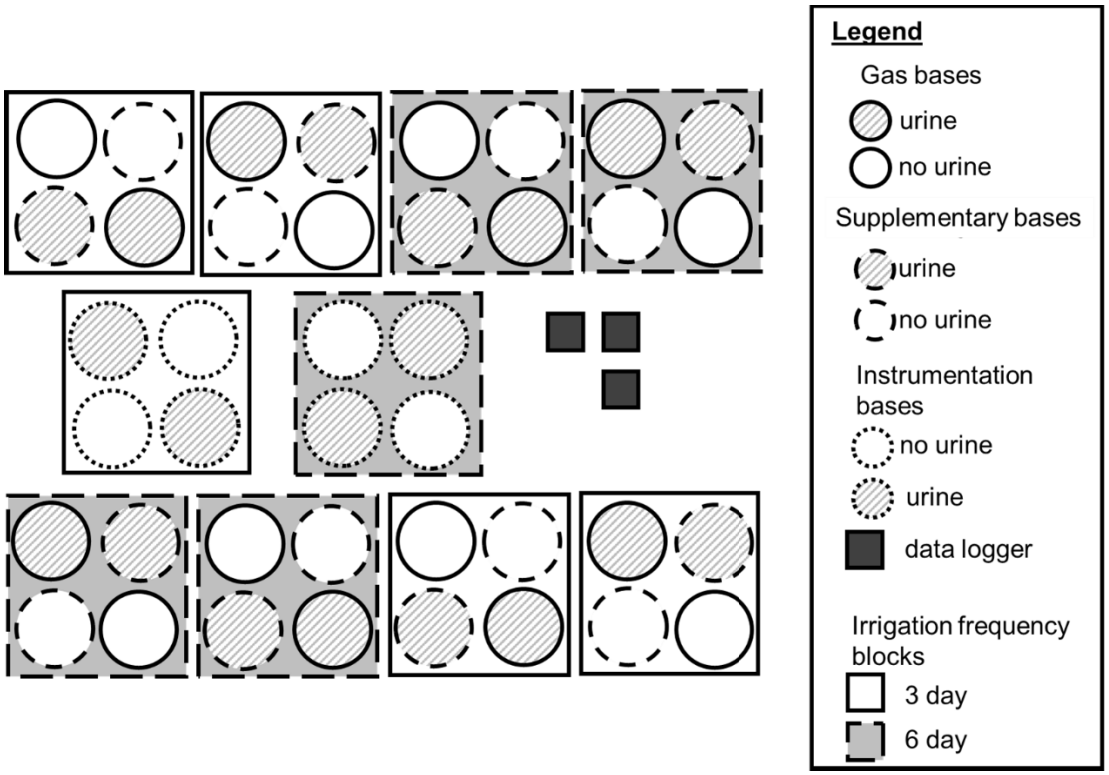
Ln, natural log

ʎ, indicates a box cox transformation was used, where the number presented is the optimal lambda

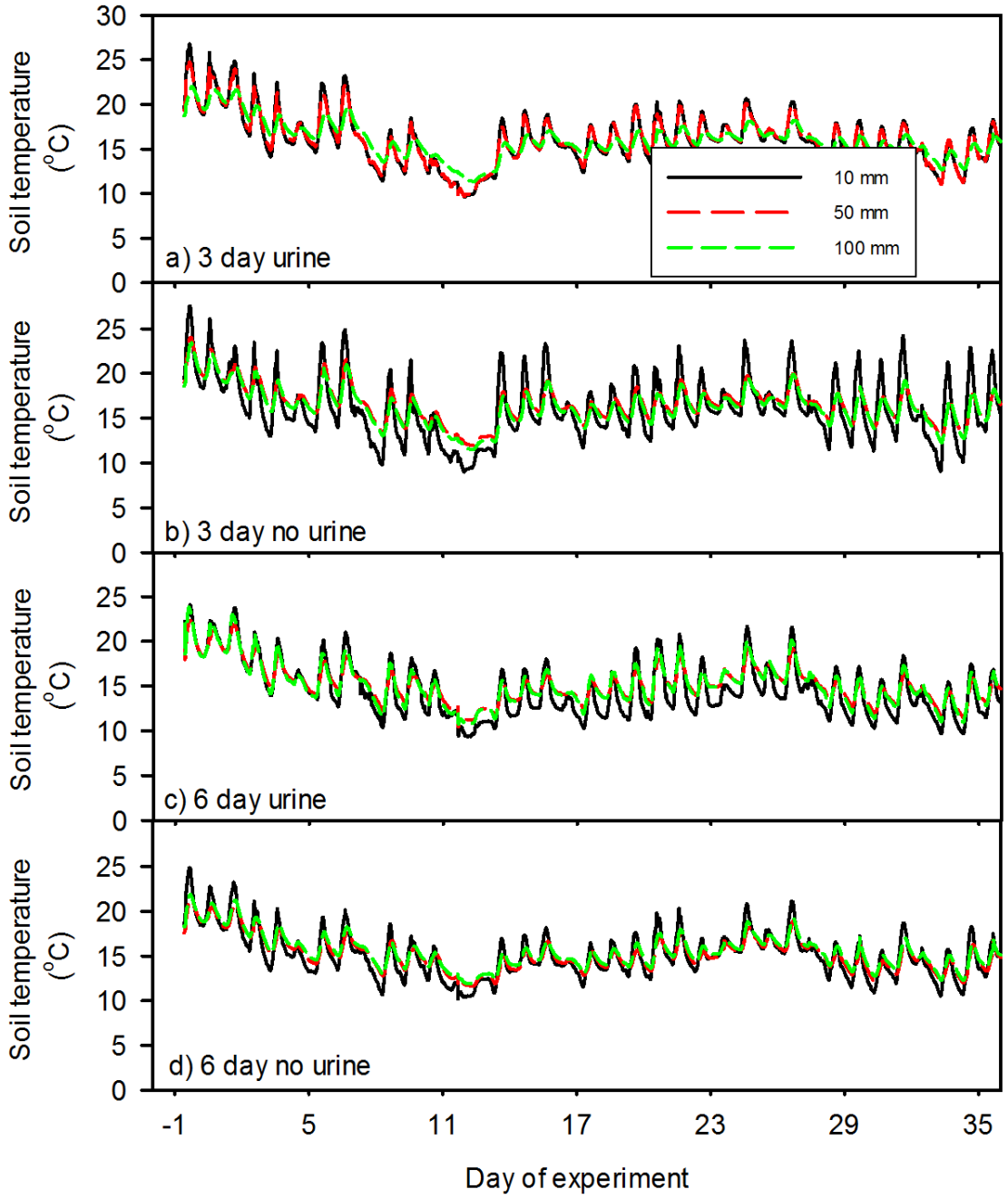
(value +1), indicates the concentration plus 1 was used for the transform to remove zeros

Supplemental Table S2 Effects of irrigation frequency and urine on mean dry matter yield (kg ha^{-1}) with the standard error of the mean in brackets from the first cut (day 16 of the experiment n = 8 for each treatment), second cut (day 35 of the experiment, n = 8 for each treatment), and total yield over the duration of the experiment (the sum both the first and second cut, n = 16 for each treatment).

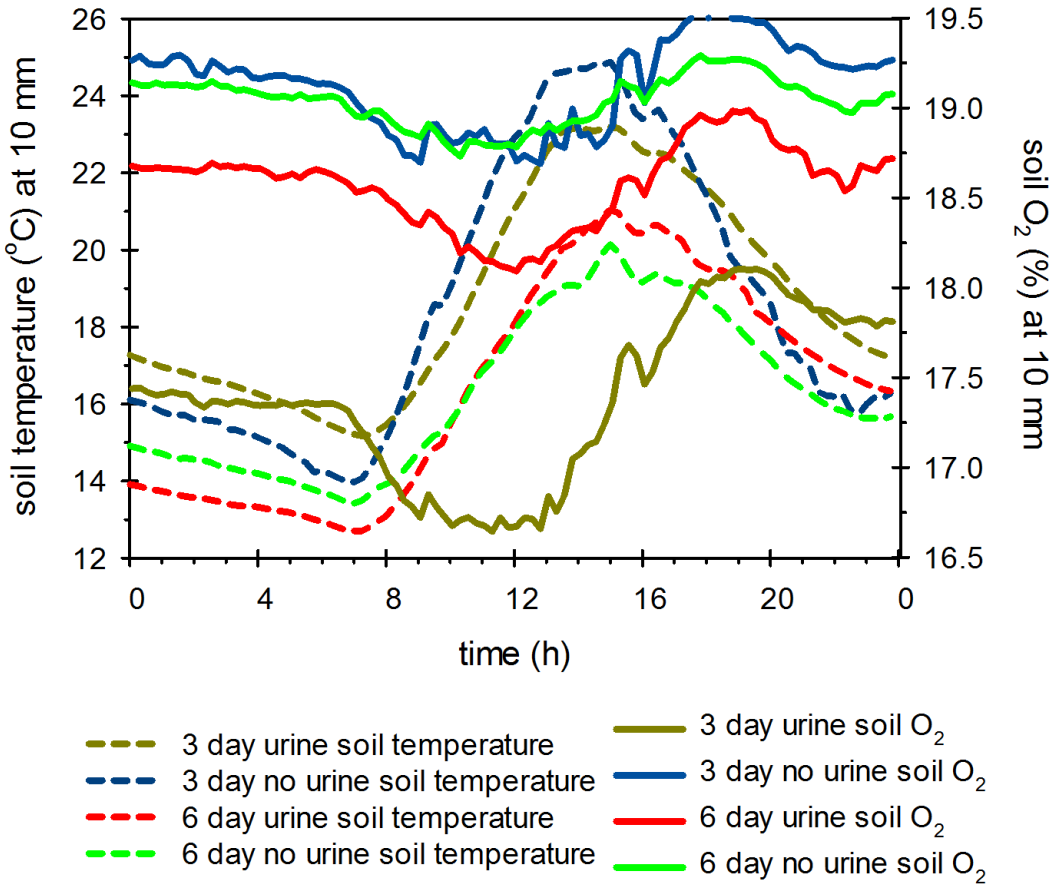
time of harvest	treatment	mean yield (kg ha^{-1}) (± SEM)	mean % N (± SEM)
first cut	urine	1479.1 (84.7)	4.45 (0.36)
	no urine	1220.9 (99.8)	4.11 (0.31)
	3 day	1377.7 (116.3)	4.28 (0.31)
	6 day	1322.2 (90.2)	4.31 (0.40)
second cut	urine	2275.0 (89.7)	4.20 (0.27)
	no urine	1413.9 (154.2)	4.10 (0.26)
	3 day	1968.5 (183.6)	3.74 (0.20)
	6 day	1720.3 (216.0)	4.57 (0.17)
total yield	urine	3754.0 (146.2)	
	no urine	2634.7 (227.0)	
	3 day	3346.2 (183.6)	
	6 day	3042.5 (216.0)	



Supplemental Fig. S1. A schema showing the relative location of the gas collars for gas sampling, supplementary bases for soil sampling and instrumentation bases for instrumentation placement, in the experimental plot. The width of each collars was 0.5 m in diameter with an area of 0.19635 m^2.



Supplemental Fig. S2. The soil temperature time series at each soil depth from which is was measured from each treatment.



Supplemental Fig. S3. An example of diurnal cycle of soil temperature and soil oxygen (O_2) at 10 mm depth for each treatment on Day of the experiment 6, showing an example of the relationship between the two variables.

Chapter 5.

Nitrous Oxide Fluxes and Soil Oxygen Dynamics of Soil Treated with Cow Urine

Chapter 4, Experiment 1, showed that D_P/D_O could explain variability in the N_2O fluxes. However, due to the free draining nature of the soil, and because the hydrological inputs were controlled, there was little variability in hydrology induced changes to soil O_2 or D_P/D_O. This experiment aims to explore how well soil O_2 measurements, and soil O_2 expressed as modelled D_P/D_O, explain N_2O fluxes in a poorly drained soil under variable hydrological conditions. This has been submitted to Soil Science Society America Journal (manuscript ID S-2016-09-0277-OR) and is presented in manuscript format.

5.1 Abstract

Ruminant urine deposition onto pasture creates hot-spots where emissions of nitrous oxide (N_2O) are produced by aerobic and anaerobic microbial pathways. However, limited measurements of *in situ* soil oxygen (O_2)-N_2O relationships hinder the prediction of N_2O emissions from urine-affected soil. This study tested whether soil O_2 concentration or relative diffusivity of O_2 (D_p/D_o) could explain N_2O emissions from urine patches. Using a randomized plot design, N_2O emissions were measured daily from a perennial ryegrass (*Lolium perenne* L.) pasture for 56 days following bovine urine deposition to an imperfectly drained silty loam soil. Soil O_2, volumetric water content, pH, conductivity, and extractable nitrogen (N) and carbon (C) were measured in urine-amended and non-amended soil. Values of water-filled pore space (WFPS) and D_P/D_O were modeled. When data from treatments were pooled together, daily mean D_P/D_O explained 73% of the total variance in mean daily N_2O flux, compared to 65, <60, and <20% for WFPS, O_2 and other measured variables, respectively. Soil pH, O_2, volumetric water content, WFPS and D_P/D_O all explained more of the variance in the urine-amended compared to the non-amended soil. Daily N_2O fluxes increased substantially at D_P/D_O values around 0.006, which was consistent with past laboratory studies. These results demonstrate for the first time an O_2 diffusion threshold for elevated N_2O fluxes in the field, expressed as $D_P/D_O \approx 0.006$. Further studies should examine the consistency of this threshold under varying N and C substrates and a range of soil pH.

Key words: Relative soil gas diffusivity, greenhouse gas emissions

5.2 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) that contributes to climate change, and it is projected to be the dominate ozone-depleting substance emitted in the 21st century (Ravishankara et al. 2009). Increases in atmospheric N_2O concentrations are linked to nitrogen (N) based fertilizer inputs and excretal returns from grazing ruminant livestock to agricultural soils. High inputs of N from these sources can cause soil N concentrations to be greater than plant requirements. This excess soil N is available for microbial processes such as nitrification, denitrification and nitrifier-denitrification, the latter two processes dominate the production of N_2O (Wrage et al. 2001, Davidson 2009, Kool et al. 2010, Zhu et al. 2013).

Nitrous oxide is produced from denitrification and nitrifier-denitrification when soil O_2 is low (Goreau et al. 1980, Firestone and Davidson 1989, Venterea 2007, Zhu et al. 2013). Soil O_2 distribution *in situ* is variable (Butterbach-Bahl et al. 2013), so even soils considered aerobic can have anaerobic microsites where N_2O production may occur (Robertson et al. 1989, Laughlin and Stevens 2002, Müller et al. 2004). Soil O_2 concentrations, and the distribution of soil O_2, are influenced by chemical reactions, microbial activity, and hydrological events. For example, urine deposition onto soil from grazing ruminant animals increases soil water content, initiates urea hydrolysis, and increases microbial respiration rates (Uchida et al. 2008). The combination of these factors can intensify O_2 depletion under a urine patch (Norton and Stark 2011) and in turn, increase soil-to-atmosphere N_2O emissions (Owens et al. 2016). Increasing soil moisture content alone can reduce soil O_2 concentrations because water impedes the diffusion of O_2 into and through soil thereby restricting soil O_2 distribution in soil (Farquharson and Baldock 2008). The relative volumes and distribution of water and O_2 in soil are regulated by soil properties, including structure (Farquharson and Baldock 2008), texture (Schjønning et al. 1999), and soil pore size distribution (Horn and Smucker 2005). The extent to which chemical, hydrological and soil physical properties influence soil O_2, and in turn, influence surface N_2O emissions, is difficult to quantify. Previous work has added labelled ^{18}O- and ^{15}N-labeled compounds to evaluate scenarios of O_2 exchange in various microbial pathways (Kool 2009) but few studies have simultaneously measured both N_2O emissions and soil O_2 concentrations in the field (Simojoki and Jaakkola 2000, Owens et al. 2016).

Diffusion of O_2 in and through soil can be modeled or inferred using soil physical and hydrological data. Relative soil gas diffusivity of O_2 (D_P/D_O) describes the rate of gas diffusion within soil (D_P) relative to free air (D_O). It can be calculated as a function of relative air-filled porosity - which is derived from soil bulk density, soil particle density, and volumetric water content - and total porosity (Schjønning et al. 1999, Moldrup et al. 2001). Relative soil gas diffusivity is a good predictor of O_2 diffusion through a soil because it accounts for the interaction between soil bulk density, the resulting pore size distribution, and the ensuing soil moisture content (Moldrup et al. 2013). Relative soil gas diffusivity has been shown to explain the rapid increase in rates of N_2O fluxes under controlled laboratory conditions when NO_3^- and C are available, with peak N_2O fluxes occurring at a D_P/D_O value of 0.006 following N substrate additions (Balaine et al. 2013, Balaine et al. 2016). However, D_P/D_O may also be a valuable tool to explain N_2O fluxes *in situ*, but more data are needed to test this.

Since soil D_P/D_O has outperformed WFPS as a predictor of N_2O fluxes in controlled laboratory studies using repacked soil cores (Balaine et al. 2013, Balaine et al. 2016), this field study aimed to build on this work by relating the same concepts in the field under variable hydrological conditions. The objective of this field study was to assess how well different measures of soil moisture, soil O_2, and D_P/D_O, as well as a range of other soil chemical variables, explained N_2O emissions from a poorly drained pasture soil, with and without ruminant urine addition.

5.3 Materials and Methods

5.3.1 Study Site

The experiment was conducted at Lincoln University (-42° 38' 81.4' S, 172° 27' 63.3" E, elevation 9 m above sea level) in July-August 2014 (southern-hemisphere winter). The soil was a stone-free, imperfectly drained Wakanui Mottled Immature Pallic Silty Loam Soil in the New Zealand Soil Classification (Hewitt 2010), or an Endoaquept in the USDA Classification (Soil Survey Division Staff 1999). The experimental plot was situated on an established, long-term unfertilized pasture sown with perennial ryegrass. Previously, the pasture has been grazed by sheep. Currently, the area is an established research station, and has not been grazed for 10 years. During the growing season, between October and April, the pasture is mown ≈ once a month.

5.3.2 Experimental Design

The experiment used two treatments replicated four times. The urine treatment applied bovine urine once at the beginning of the experiment, subsequently referred to as "day of experiment" zero ("DOE" 0). The urine was collected from cows grazing perennial ryegrass and white clover (*Trifolium repens L.*) pasture. A subsample of the urine was immediately analyzed after urine collection on a CN elemental analyser (Elementar Vario-Max CN Elemental Analayser, Elementar GmbH, Hanau, Germany) to determine total N content. The total N content of the urine was increased from 4.9 g N L^{-1} to 7.5 g N L^{-1} using urea [$CO(NH_2)_2$], the dominate N source in ruminant urine, and then the urine was stored in a sealed container with no headspace. Two L of urine (equalling 10.2 mm of water) was applied within each chamber area for the urine treatment at a rate of 750 kg N ha^{-1}, a typical cattle urine deposition event (Haynes and Williams 1993). The urine treatment was applied to the soil within 24 hours of urine total N determination. Nothing was applied to the soil in the no urine treatment. This was done to mimic actual field conditions of an area that is not affected by urine deposition.

A 4 by 6 m experimental area was subdivided into plots for gas sampling, manual sampling of soil, and for installation of automated instrumentation (Supplemental Fig. S1). Within each of the plots, circular stainless steel gas-flux chamber bases (0.196 m^2) were installed for gas sampling, to delineate areas soil sampling, and to distinguish areas where the automated instrumentation was installed (Supplemental Fig. S1). Each chamber base was inserted into the soil DOE -21 to a depth of 100 mm.

The experimental plot was covered with a tunnel house (Torto, Hamilton, New Zealand) between DOE -2 and 20. The original goal was to exclude precipitation so that soil water content could be controlled via irrigation. Between DOE 2 and 20, there was a total of 48 mm of precipitation. This resulted in surface flooding of the experimental area between DOE 19 and 22. Because of this, the tunnel house was removed on DOE 20. Periodically, the soil was manually irrigated in an attempt to decrease soil O_2 concentrations. On DOE 6, 11, and 13, 6 mm of irrigation was applied. On DOE 29, 14 mm of irrigation was applied, and 20 mm of irrigation was applied on DOE 49, 50, and 51 (Figure 5.1 a). In addition to irrigation, 16 mm of rain fell between DOE 21 and 56 directly onto the experimental plot.

5.3.3 Nitrous Oxide Fluxes

Soil-to-atmosphere N_2O fluxes were measured daily from DOE -1 to DOE 56, except between DOE 49 and 51, using non-steady state vented and insulated chambers (headspace volume = 19.63 L). To attain fluxes representative of the daily average, sampling occurred between 10:00 am and noon local time (van der Weerden et al. 2013). Four N_2O samples were taken at 0, 15, 30, and 45 min following placement of the chamber lids onto the chamber base. Gas (9 mL) was collected and transferred to 6 mL pre-evacuated (-1 atm) glass Exetainers® (Labco Ltd., Lampeter, United Kingdom) using a syringe fitted with a 3-way stop cock. Gas samples were analyzed with an automated gas chromatograph equipped with an electron capture detector (SRI 8610c GC, SRI Instruments, Torrance, California, USA), as previously described (Clough et al. 1996). The detection limit of the gas chromatograph was 0.01 µL L-1 and the furnace temperature was 310°C. Nitrous oxide concentrations were converted to mass per volume concentration using the ideal gas law and air temperature at the time of sampling. Flux calculations used the change in N_2O concentration over time, along with the chamber volume and area. Initially, both quadratic regression (Wagner et al. 1997) and linear regression were used to determine the change in N_2O concentration. The quadratic regression fluxes were evaluated using the LINEST function in Microsoft Excel (version 2013). Flux calculations used quadratic regression unless the second derivative of the quadratic regression was ≥ 0 (Venterea et al. 2009, Venterea 2013). All measured fluxes were above the detection limit (Parkin et al. 2012). Of the 408 flux calculations, 34% were calculated using quadratic regression and 66% using linear regression. A correction factor was applied to account for chamber-induced errors (Venterea 2010). This required knowing the soil bulk density within each gas chamber base. Soil bulk density was determined from an average of three intact soil cores (height = 75 mm, i.d. = 75 mm) which were removed at the end of the experiment from the area within each gas-flux measurement area.

Cumulative N_2O emissions from each chamber were determined by summing the daily N_2O flux estimates. Nitrous oxide fluxes from days without a flux measurement were derived using linear interpolation. The emission factor was determined by subtracting the cumulative N_2O emissions in the no urine treatment from the cumulative N_2O emissions in the urine treatment, then dividing the sum by the rate of urine N applied, and expressing this as a percentage of N applied (de Klein et al. 2003).

5.3.4 Soil and Environmental Variables

Precipitation (mm) data were acquired from a Lincoln weather station 2 km north-west of the experiment site (Broadfield, Lincoln, -43° 37' 57.2" S, 172° 28' 22.4" E). Environmental instrumentation was installed in the center of the experimental plot within urine and no urine treatments (Supplemental Fig. S1). Soil temperature sensors (107 temperature sensor, Campbell Scientific, Logan, UT, USA) and volumetric water content (θ_v) sensors (CS 616 Reflectometer, Campbell Scientific, Logan, UT, USA) were installed horizontally into the soil at 50 mm depth. Soil O_2 sensors (SO-110, Apogee Instruments, Logan, UT, USA) were installed vertically at 10, 50, and 100 mm soil depths in both treatments. Two soil O_2 sensors were installed in each treatment and soil depth, one with the diffusive head attached and one without the diffusive head. The purpose of the different diffusive head configurations was to measure different sized areas within the soil. The motivation was that these different measurement areas may show different soil O_2 concentrations and dynamics in response to changes to soil moisture or urine deposition; the smaller measurement area captured from the sensors without the diffusive head may reveal a relatively finer resolution measurement of soil O_2 compared to the sensors with the diffusive head. Data from the O_2 sensor without the diffusive head at the 50 mm depth in the no urine treatment are not reported because the sensor malfunctioned. The instrumentation was powered and controlled by two data loggers and a multiplexer (CR3000, CR1000, AM416, Campbell Scientific, Logan, UT, USA). Samples were taken every 15 min from DOE -1 (0:00 July 2, 2014) until DOE 56 (12:00 August 27, 2014). Manual tensiometer readings (2900F1 Quick Draw Tensiometer, Soilmoisture Equipment Corp. Santa Barbara, CA, USA) were also taken once daily, from within each gas chamber base at two depths (≈20 and ≈70 mm), to measure soil matric potential (Ψ) from DOE 19 to DOE 56, during and after surface flooding. Soil WFPS was calculated using measured θ_v at 50 mm soil depth (Linn and Doran 1984). Soil bulk density was measured as described above. The capillary rise equation (Supplementary Table S1), which can be used to any given ψ value to determine the an equivalent pore radius that remains full of water at that ψ value (Scott, 2000; Hillel,2004), is used to determine the size of soil pore which was water-filled at the minimum measured ψ. Calculations used in this paper are available in Supplemental Table S1. Pasture was harvested to ≈50 mm height using hand held shears on DOE -1, 26, and 56 to simulate grazing.

The soil sampling areas within the manual sampling chambers and the instrumentation chambers for each treatment were treated the same as the soil in the gas sampling chambers. The soil was sampled from these areas to a depth of 70 mm using an auger. Soil was sampled every six days

between DOE -1 and 56 for soil chemical analysis. Soil pH was determined by mixing 10 g of air-dried soil with 25 mL deionized water (DI) and the solution was measured (SevenEasy, Mettler Toledo, Port Melbourne, Australia) after 12 h of settling (Blakemore et al. 1987). Conductivity was determined by combining 10 g dry weight equivalent of soil with 50 mL of DI, mixing for 30 min and measuring (SevenEasy, Mettler Toledo, Port Melbourne, Australia) following 5 min of centrifuging at 1500 rpm (Blakemore et al. 1987). Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were determined by extracting 4 g dry weight equivalent of soil with 40 mL 2M KCl. Samples were mixed for 1 h, centrifuged for 20 min (2000 rpm), and gravity filtered through Whatman °42 filters (Blakemore et al. 1987). Extracts were frozen until flow injection analysis (FIAstar 5000 Analyzer, FOSS Analytical, Hilerød, Denmark).

Extractable cold water carbon (CWC), indicative of water soluble C, was extracted by combining 3 g dry weight equivalent of soil and 30 mL of DI followed by 30 min of mixing, centrifuging for 20 min (3500 rpm), and filtering through Avantec 5C filters (Ghani et al. 2003). Then the soil was extracted again to obtain hot water extractable carbon (HWC), which is related to microbial biomass. After adding DI as before, the soil-DI mixture was placed in a hot water bath at 80°C for 16 h before mixing, centrifuging, and filtering as noted above (Ghani et al. 2003). The CWC and HWC samples were frozen after extraction until analysis with a Total Organic Carbon Analyzer (TOC 5000A, Shimadzu, Sydney, Australia).

The Structure-dependent Water-induced Linear Reduction (SWLR) model (Moldrup et al. 2013) was used to calculate D_P/D_O values using the previously measured soil bulk density from within the chambers, and the daily air-filled porosity (Supplemental Table S1).

5.3.5 Data Analyses

Unless otherwise stated, data analyses were performed using Minitab (Minitab Inc. version 17 2010) with parametric statistics. Data were transformed (Supplemental Table S2) if needed. Analysis of urine treatment effects on overall means included only data collected after urine application (from DOE 1 onward). If data were transformed, conclusions were drawn from the analysis on the transformed scale. Figures present untransformed data unless otherwise noted.

A linear mixed model run in SPSS (IBM Corp. version 20.0 2011) using a significance criteria of 0.05 was used to test for treatment effects on mean daily N_2O emissions. This model was used to compensate for repeated measures and heterogeneity of variance between treatments. A

heterogeneous first-order autoregressive covariance structure was used for the repeated measures. The effect of urine, DOE, and urine x DOE were treated as fixed effects, and DOE as a repeated measure. Tukey's multiple comparisons was used as a post hoc test (Steel et al. 1997).

A general linear model (GLM) was used to test for treatment effects on overall means for all soil and environmental data (except Ψ). For NH_4^+, NO_3^-, HWC, CWC, soil pH, and conductivity, the urine, DOE, and urine x DOE were treated as fixed effects. For overall mean soil temperature at 50 mm soil depth, θv at 50 mm soil depth, WFPS, soil O_2 at 10 and 100 mm soil depth from sensors with and without the diffusive heads, and modeled D_P/D_O, only urine and DOE were treated as fixed effects. Tukey's multiple comparison test at $P < 0.05$ was used for post hoc tests (Steel et al. 1997). A two-sample student t-test was used to test for differences in soil bulk density and total porosity between the treatments.

Least squares linear regression was used to evaluate the relationships between daily N_2O fluxes, and the daily mean measured and calculated factors noted above.

5.4 Results

5.4.1 Soil and Environmental Variables

The soil bulk density of the urine treatment was higher than from the no urine treatment ($P = 0.086$). Soil bulk density averaged 1.01 (±0.054 SEM; n=4) and 1.24 Mg m^{-3} (±0.098 SEM; n=4) in the no urine and urine treatments, respectively. Total porosity was lower from urine treatment compared to the no urine treatment ($P = 0.086$), averaging 62 (± 2.1 SEM; n=4) and 53% (± 3.7 SEM; n=4) in the no urine and urine treatments, respectively.

Air temperature averaged 7.2°C over the course of the experiment, with minimum and maximum values of -2.4°C and 20.6°C, respectively. Soil temperatures followed a diel cycle with overall mean soil temperatures ranging from 7.2 to 7.6°C, and there was no difference between the two treatments.

There was no difference in overall mean pH between the treatments. Soil pH increased after urine deposition but was lower in the urine treatment compared to the no urine treatment at the end of the experiment (Figure 5.1 a). Overall mean soil conductivity, NH_4^+ concentrations, and NO_3^- concentrations were 211 ($P < 0.001$), 95 ($P = 0.016$), and 80% ($P < 0.001$) greater in the urine treatment compared to the no urine treatment (Figure 5.1 b-d). Overall mean CWC (Figure 5.1 e)

and HWC (Figure 5.1 Fig. 1 f) concentrations were not affected by urine. All extractable soil environmental factors varied with DOE (Figure 5.1).

Rapid increases in θ_v occurred following urine application, heavy irrigation, and precipitation (Figure 5.2 a, b). Surface flooding resulted in high θ_v at 50 mm soil depth in both treatments between DOE 19 and 22 (Figure 5.2 b). Overall daily mean θ_v was 6% higher in the urine treatment compared to the no urine treatment ($P < 0.001$). The highest N_2O fluxes were observed between 0.70 and 0.80 m^3 m^{-3} WFPS (Figure 5.2 c). The overall daily mean WFPS was 17% higher in the urine treatment compared to the no urine treatment ($P < 0.001$), consistent with the relatively higher bulk density in the urine treatment. Matric potential ranged from ≈0 kPa during surface flooding, to a minimum of -11 kPa on DOE 45 (Figure 5.2 c). There was some spatial variability in Ψ. At each depth, the surface flooding differed by a maximum of 6 kPa between gas chambers on each day (Supplemental Fig. S2).

At 10 mm soil depth, the overall mean soil O_2 concentration was higher in the urine treatment compared to the no urine treatment by 4.5 ($P < 0.001$) and 6.1% ($P < 0.001$) with and without the diffusive head present, respectively. Soil O_2 concentrations decreased following urine deposition for a period of ≈24 h at both 50 and 100 mm soil depths regardless of the presence or absence of the diffusive head (Figure 5.2 e-f). Minimum soil O_2 concentrations at 50 and 100 mm soil depths occurred following surface flooding, and prior to drainage on DOE 23, regardless of O_2 sensor diffusive head configuration (Figure 5.2 e-h). At 100 mm depth, the overall mean soil O_2 concentration was lower in the urine compared to the no urine treatment by 27% ($P < 0.001$) when the diffusive head was present, and 17% ($P < 0.001$) when the diffusive head was absent, respectively.

In situ modeled D_P/D_O decreased when θ_v increased (Figure 5.2 i). Due to the higher average bulk density and lower average porosity in the urine treatment compared to the no urine treatment, the overall mean D_P/D_O was 149% lower in the urine treatment ($P < 0.001$).

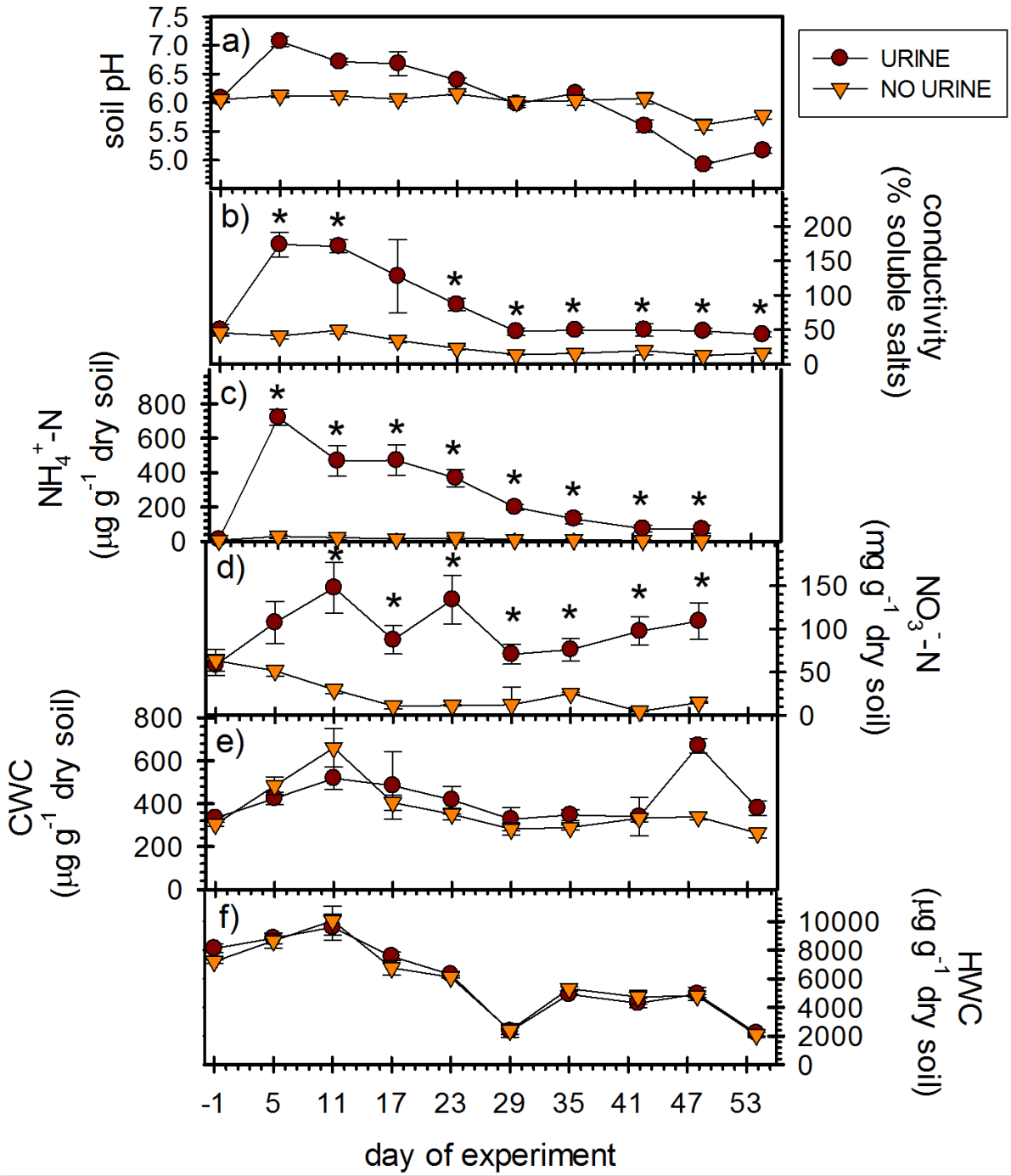


Figure 5.1 Means and standard error of the means (±SEM, n = 4) of the soil chemical data over time, a) soil pH, b) conductivity, c) ammonium (NH_4^+-N), d) nitrate (NO_3^--N), e) cold water carbon (CWC), and f) hot water carbon (HWC), where the asterisks (*) indicate a significant difference between the treatments at $P < 0.05$.

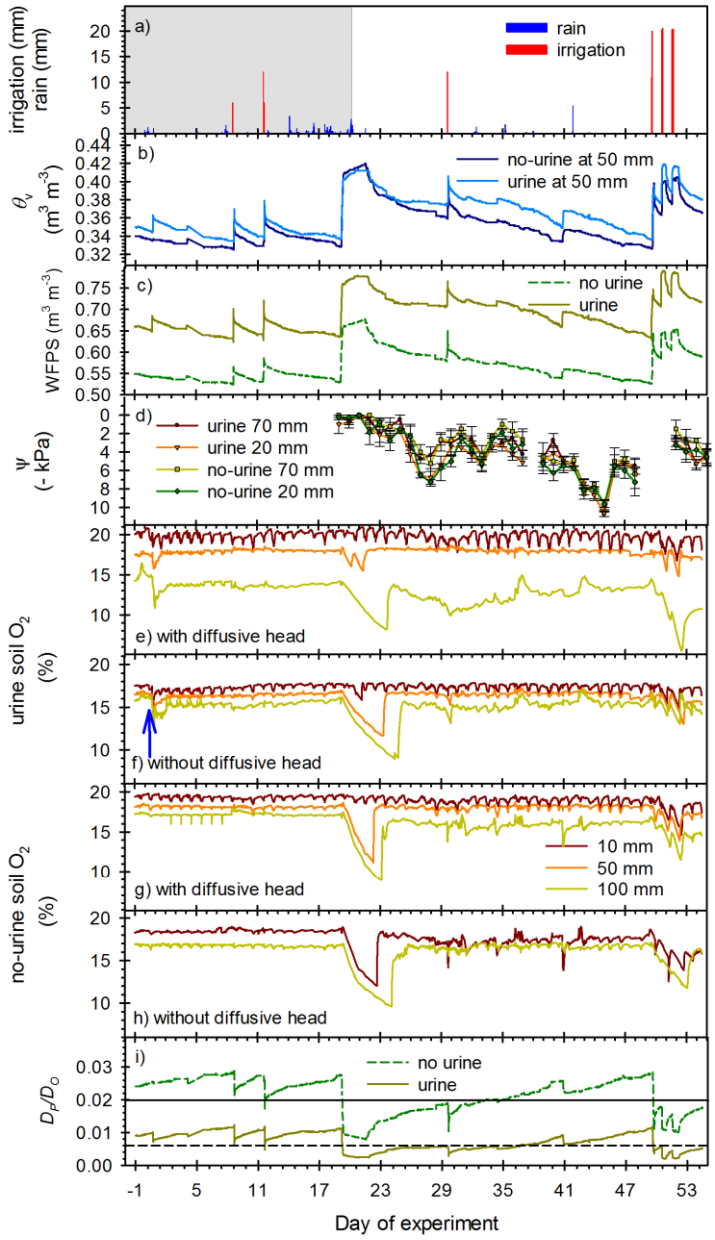


Figure 5.2 (a) Rain (blue) and irrigation (red) with the grey shaded area representing when the experimental plot was covered with the tunnel house, (b) the volumetric water content (m^3 m^{-3}) over time from the urine and no urine treatment at 50 mm soil depth, (c) water-filled pore space over time from urine and no urine, and (d) daily tensiometer readings from the gas collars (n = 4, ±SEM) from the urine and no urine treatment, at 20 and 70 mm soil depths. Soil oxygen (O_2) from the urine treatment at 10, 50, and 100 mm soil depths from the sensors with the diffusive head (e) and without the diffusive head (f), and soil O_2 from the no urine treatment at 10, 50, and 100 mm soil depths from the sensors with the diffusive head (g) and without the diffusive head (h). Relative soil gas diffusivity (i) modeled using the SWLR model (Moldrup et al., 2013) where the solid line marks 0.02 and the dashed line marks 0.006. The arrow indicates the time of urine application.

5.4.2 Nitrous Oxide Fluxes

Daily average N_2O fluxes were 16 times greater from the urine treatment compared to the no urine treatment ($P < 0.001$), and there was a significant urine-by-DOE interaction effect ($P < 0.001$, Figure 5.3). Differences by DOE were associated with increased N_2O fluxes following urine deposition, surface flooding (DOE 19 - 22), and heavy irrigation on DOE 52 (Figure 5.2 Fig. 2 and Figure 5.3). Fluxes of N_2O increased as D_P/D_O declined towards ≈ 0.006 and negative N_2O fluxes were also observed on DOE 53 and 54, also at a D_P/D_O value of ≈ 0.006 (Figure 5.3, Figure 5.4 c). Cumulative N_2O emissions were also greater from the urine treated soil ($P = 0.016$), with an emission factor of 2.1%.

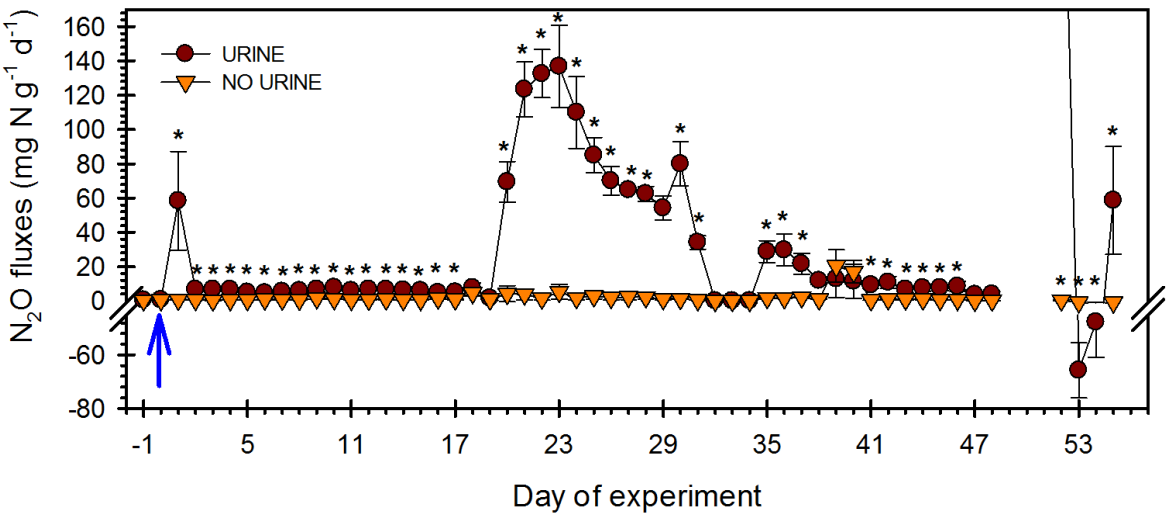


Figure 5.3 Daily mean N_2O fluxes (± SEM, n = 4) from the urine and no urine treatment over time where the asterisks (*) represents a difference between the treatments at $P < 0.05$. On DOE 52, mean fluxes for the urine treatment go up to 330.3 mg N m^{-2} d^{-1} (±SEM 123.2). The arrow indicates the time of urine application.

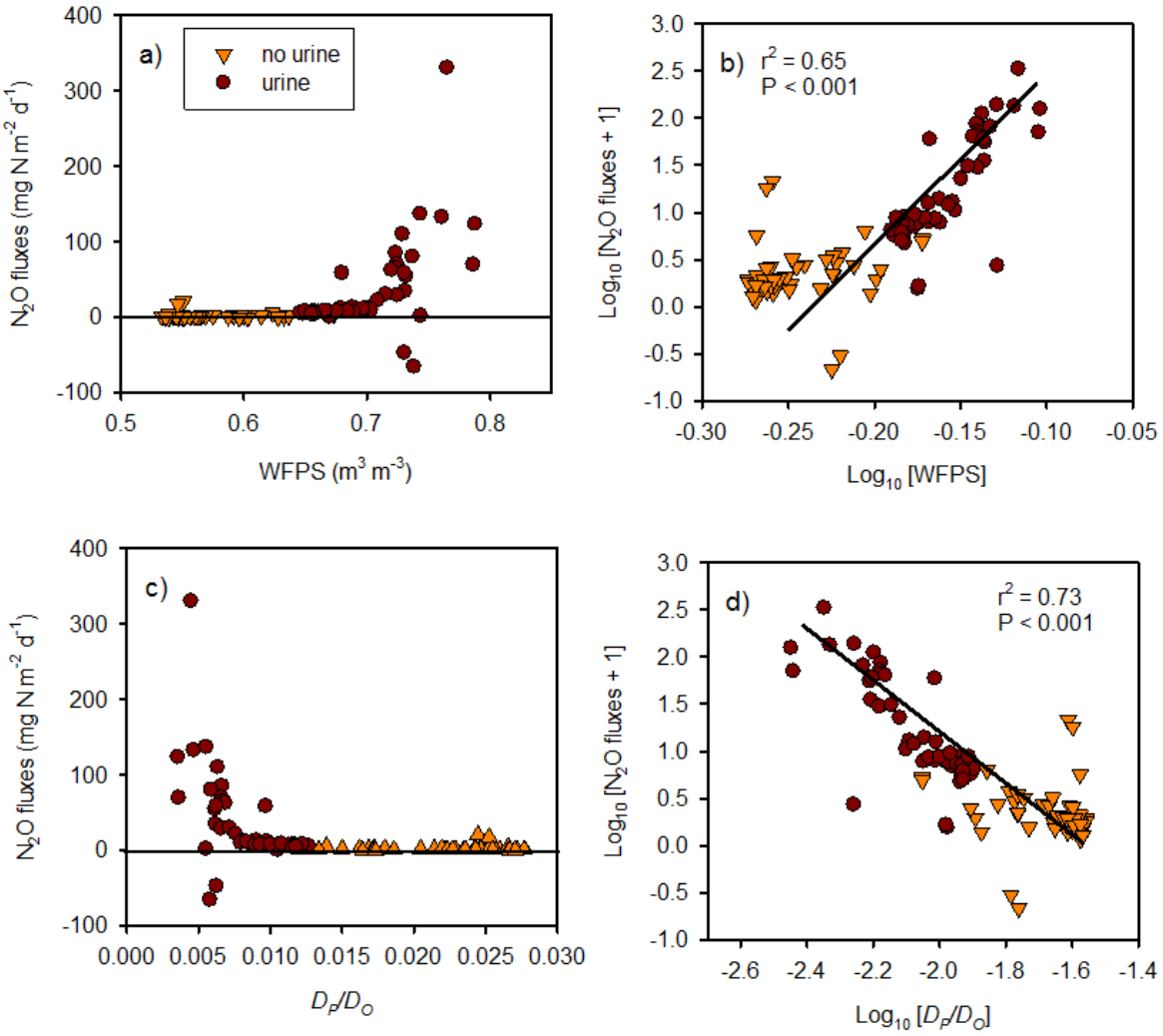


Figure 5.4 The daily average N_2O fluxes and (a) water-filled pore space (WFPS) and (c) relative soil gas diffusivity (D_P/D_O), and (b, d) the same data represented with both variables log-transformed, and a linear regression through both the urine and no urine treatment data.

Individually, soil temperature, NO_3^-, NH_4^+, HWC, CWC, soil pH, and conductivity explained ≤ 17% of the variability in N_2O fluxes when all data were considered, ≤ 15% of the variability in N_2O fluxes when only the urine data were considered, and ≤ 9% of the variability in N_2O fluxes when only the no urine data were considered (Table 5.1).

When all data were pooled, soil O_2 explained ≤ 59% of the variability in N_2O fluxes (Table 5.1). All O_2 data, except that measured at 50 mm with the diffusive head, were significantly related with N_2O fluxes. However, negative relationships were observed between O_2 and N_2O fluxes at 50 and 100 mm, and a weaker but positive relationship was observed between O_2 and N_2O fluxes at 10 mm. In only the urine treatment, daily average soil O_2 at 100 mm soil depth explained ≤ 69% of the

variability of N_2O both with and without the diffusive head (Table 5.1). Soil O_2 was not significantly related to N_2O fluxes in the no urine treatment fluxes (Table 5.1).

Volumetric water content, WFPS, and D_P/D_O were significantly related to N_2O fluxes within individual treatments, and when both treatments were pooled (Table 5.1). The strongest relationship in the urine treatment was between WFPS and N_2O fluxes, but when both treatments were considered together, the strongest relationship was between D_P/D_O and N_2O fluxes (Table 5.1).

There was an increase in N_2O fluxes associated with the increase in WFPS. However, the high N_2O fluxes occurred over a WFPS range of ≈0.70 to ≈0.80 $m^3\ m^{-3}$ (Figure 5.4). The increase in N_2O fluxes associated with a decrease in D_P/D_O, showed that the high N_2O fluxes occurred over a relative narrow D_P/D_O range of 0.004 to 0.006 (Figure 5.4).

Table 5.1 P-values and regression analyses relating daily average N_2O fluxes and daily average environmental variables. The "+" or "-" sign in front of the r^2 value represents whether the relationship between the variables is positive or negative, respectively.

Regression								
Variable	Units	Depth (mm)	all data		urine		no urine	
			P	r^2	*P*	r^2	*P*	r^2
Ammonium	(µg N g^{-1} dry soil)	0-70	0.000	+ 0.17	0.312	+ 0.03	0.353	+ 0.03
Nitrate	(µg N g^{-1} dry soil)	0-70	0.002	+ 0.12	0.251	+ 0.04	0.469	- 0.02
Hot water carbon	(µg g^{-1} dry soil)	0-70	0.089	+ 0.04	0.097	+ 0.07	0.808	+ 0.00
Cold water carbon	(µg g^{-1} dry soil)	0-70	0.852	+ 0.00	0.959	- 0.00	0.995	+ 0.00
pH	(---)	0-70	0.001	+ 0.14	0.015	+ 0.15	0.057	+ 0.09
Conductivity	(% soluble salts)	0-70	0.088	- 0.04	0.267	- 0.03	0.970	+ 0.00
Temperature	(°C)	50	0.705	- 0.02	0.976	+ 0.00	0.360	+ 0.02
O_2 with diffusive head	(%)	10	0.000	+ 0.28	0.287	+ 0.03	0.906	+ 0.00
		50	0.564	+ 0.04	0.522	- 0.09	0.277	- 0.03
		100	0.000	- 0.59	0.000	- 0.69	0.062	- 0.08
O_2 without diffusive head	(%)	10	0.000	+ 0.16	0.239	- 0.03	0.111	- 0.06
		50	0.000	- 0.41	0.00	- 0.27	N/A	N/A
		100	0.000	- 0.56	0.000	- 0.43	0.181	- 0.04
Volumetric water content	(m^3 m^{-3})	50	0.000	- 0.35	0.000	- 0.5	0.022	- 0.11
Water-filled pore space	(m^3 m^{-3})	50	0.000	+ 0.65	0.00	+ 0.82	0.027	+ 0.11
Relative soil gas diffusivity	(---)	50	0.000	- 0.73	0.00	- 0.65	0.023	- 0.11

5.5 Discussion

Nitrous oxide fluxes from the urine treatment in the current study, as well as the emission factor, were similar to previously reported values from poorly drained pasture soils (Luo et al. 2008, Kelliher et al. 2014). Urine application onto a pasture soil induces hydrolysis reactions, which are followed by biological nitrification and denitrification (Baral et al. 2014). These processes can increase inorganic N concentrations and N_2O emissions (Orwin et al. 2010, Taghizadeh-Toosi et al. 2011, Owens et al. 2016). The weak relationships observed between all environmental variables and daily N_2O fluxes in the no urine treatment were due to limited inorganic N substrate availability for N_2O production in that treatment. Higher N_2O emissions from the urine treatment were due to greater substrate availability (Figure 5.1). However, soil nutrient concentrations were not correlated with N_2O fluxes (Table 5.1). Instead, variables pertaining to soil aeration, including soil O_2 measurements, WFPS, and D_P/D_O explained the variability in N_2O fluxes when N was not limiting.

Oxygen concentrations are a proximal controller of the microbial processes responsible for N_2O production (Knowles 1982, Firestone and Davidson 1989, Wrage et al. 2001). While soil O_2 often related with N_2O fluxes in the current study, especially in the urine treatment (Table 5.1), neither of the diffusive head configurations used with the soil O_2 sensor consistently explained N_2O fluxes in all treatments. The results suggest that both O_2 sensor configurations captured bulk changes to bulk soil O_2 concentrations. For there to be a consistent relationship between N_2O fluxes and soil O_2 when substrates are not limited, there must be a measure of soil O_2 that correlates with the physical scale of the microbial processes producing N_2O in the soil. If N_2O is produced in anaerobic microsites, a measure of soil O_2 at the microscale level is needed, and the sensors need to be measuring at the same depth of N_2O production. We suspect that both diffusive head configurations measured an area that was too large, and lacked the resolution to observe O_2 dynamics at the soil macropore-micropore scale (<0.2 μm to ca. > 600 μm), which were significant to N_2O production.

Nitrous oxide fluxes increased by 1 to 3 orders of magnitude when soil O_2 decreased following heavy irrigation or surface flooding (Figure 5.3). Decreases in bulk soil O_2 can increase nitrifier-denitrification and/or denitrification rates (Goreau et al. 1980, Venterea 2007, Zhu et al. 2013). Nitrification drove the decline in NH_4^+ from DOE 5 onward in the urine treatment (Figure 5.1 c), implying that both nitrification and nitrifier-denitrification were potential sources of urine-induced N_2O fluxes during this study. During surface flooding where Ψ read 0 kPa, the soil drained to a minimum Ψ of -11 kPa - or 26.8 μm or smaller diameter soil pores still water-filled based on the

capillary rise equation - suggesting macropores and some mesopores would have drained but not the micropores (Luxmoore 1981). These water-filled microopores may have led to the development of anaerobic microsites in the soil following drainage suggesting denitrification was a potential source of N_2O fluxes (Müller et al. 2004) during this time. Further evidence of denitrification after drainage is the disparity between the decline in NH_4^+ concentrations and the increase in NO_3^- concentrations which were not equal, indicating NO_3^- was removed from the soil. While this may be partially due to NO_3^- leaching, which was not measured during this study, high N_2O fluxes coupled with high WFPS and low D_P/D_O suggest that some soil NO_3^- was denitrified and emitted as N_2O.

The relationship between WFPS and N_2O fluxes was strongest when only data from the urine treatment were considered, with less variability explained when N_2O fluxes from both treatments were pooled. Conversely, D_P/D_O explained more variability in N_2O fluxes when data from both treatments were considered (Table 5.1, Figure 5.4). This is because WFPS fails to account for the interactive effects of soil bulk density and Ψ. The difference in bulk density influenced the strength of the relationship between N_2O fluxes, and WFPS and D_P/D_O. The differences in bulk density and total porosity between the treatments occurred despite the randomization of the treatments. Bulk density was determined at the end of the experiment so this could not be accounted for during the experimental design. The differences in bulk density between the treatments highlight the issue with relying solely on the use of WFPS to explain N_2O fluxes. An integrative measure of the soil physical characteristics that directly affect soil O_2 supply, including air-filled porosity and pore size distribution is encompassed by D_P/D_O (Moldrup et al. 2013).

Nitrous oxide emissions are episodic, and high N_2O fluxes can occur over a relatively wide range of WFPS values, from 60 to 90% WFPS (Dobbie et al. 1999, Davidson et al. 2000, Müller and Sherlock 2004). This makes it difficult to predict when high fluxes will occur. In the current study, high N_2O fluxes occurred at WFPS values ranging from ≈ 0.70 to ≈ 0.80 $m^3\ m^{-3}$. This variation occurs because WFPS is not quantifying the fraction of the total soil volume that is either water- or air-filled, and so it is not a direct measure of N_2O production/consumption regulation mechanisms (Farquharson and Baldock 2008, Balaine et al. 2016). The Structure-dependent Water-induced Linear Reduction (SWLR) model from Moldrup et al. (2013) to model D_P/D_O includes provisions for variability in soil moisture content, soil texture, and soil compaction (Figure 5.4).

Controlled laboratory studies noted N_2O fluxes increased substantially as D_P/D_O lowered to a value of 0.006 (Balaine et al. 2013, Balaine et al. 2016). In the current study, N_2O fluxes also increased as

D_P/D_O declined to this value from the urine treatment, as there was available substrate. There were also negative N_2O fluxes observed on DOE 53 and 54 (Figure 5.3) which occurred below the D_P/D_O value of 0.006 (Figure 5.2 i) due to the reduction of N_2O to dinitrogen (N_2) (Chapuis-Lardy et al. 2007, Balaine et al. 2016). The enzyme responsible for the reduction of N_2O to N_2, nitrous oxide reductase (N_2OR), is highly sensitive to the presence of O_2 (Knowles 1982, Firestone and Davidson 1989, Wrage et al. 2001) and takes 33 to 48 h to synthesize after the onset of anaerobic conditions (Smith and Tiedje 1979, Dendooven and Anderson 1994). The surface flooding and wet soil conditions between DOE 20 and 35 likely primed the N_2OR pathway. After heavy irrigation between DOE 49 and 51, whereby D_P/D_O was reduced to <0.006, net N_2O consumption occurred on DOE 53 and 54 resulting in negative fluxes (Figure 5.3). The role of antecedent moisture conditions on N_2O fluxes, and prior wet conditions priming N_2OR followed by reduction in N_2O fluxes upon rewetting, has been noted in previous studies (Smith and Patrick 1983, Groffman and Tiedje 1988, Dendooven and Pemberton 1996, Bergstermann et al. 2011, Guo et al. 2014, Uchida et al. 2014, Owens et al. 2016). The concept of a D_P/D_O threshold where maximum N_2O fluxes occur, and N_2O is reduced N_2, may provide opportunities to modify soil management to minimize N_2O fluxes. If the soil were kept aerated with high D_P/D_O, then N_2O production could be limited. Alternatively, lowering D_P/D_O could encourage reduction of N_2O to N_2. Strategies could involve, for example, careful timing irrigation or ensuring soil management reduced soil compaction.

A limitation of D_P/D_O is that it did not capture chemically induced reductions in O_2 from urea hydrolysis after urine deposition (Figure 5.2 Fig. 2), and where increases in N_2O flux rates occurred. Following urea hydrolysis, the carbonate ions produced are further hydrolyzed. The ensuing re-equilibration of the inorganic-C pools results in carbon dioxide (CO_2) production occurring, and lowering O_2 concentrations, despite D_P/D_O >0.006. Similar observations were noted in the only other study to investigate this (Owens et al. 2016), where a reduction of soil O_2 and a peak in N_2O emissions were observed about two days after urine deposition, without D_p/D_o dropping below 0.006. Relative soil gas diffusivity is a physical parameter that assumes negligible biological or chemical consumption of soil O_2 (Rolston and Moldrup 2002). Future N_2O studies are needed to explore the potential interactions between D_P/D_O and different permutations of environmental conditions such as substrate supply and pH, and respiration rates, which will influence soil O_2 supply and may modify the D_P/D_O threshold of 0.006 for maximum N_2O production or reduction of N_2O to N_2 (Petersen et al. 2013).

5.6 Conclusion

In summary, soil O_2 in a poorly drained pasture decreased with hydrological events such as flooding, or chemical hydrolysis events following urine deposition. These decreases in soil O_2 coincide with rapid increases in N_2O fluxes. It was found that hydrological variables such as WFPS work well to explain N_2O emissions so long as soil properties do not vary. Relative soil gas diffusivity best explains N_2O fluxes regardless of the treatment because it can compensate for how soil properties and soil moisture interact to influence soil O_2 diffusion. These results demonstrate for the first time an O_2 diffusion threshold for elevated N_2O fluxes in the field, occurring at a value of $D_p/D_o \approx 0.006$. Further studies should examine the consistency of this threshold under varying microbial substrate and soil pH conditions.

Acknowledgements

This research was undertaken with CRI Core Funding to Landcare Research from New Zealand's Ministry of Business, Innovation and Employment. We are grateful for the help in the field provided by Neil Smith, and to the Centre for Environment and Soil Research at Lincoln University for the loaned equipment.

Supplementary material

The supplementary data includes a map of the experimental plot (Supplemental Fig. S1), a graphical representation of the spatial distribution of matric potential within the gas collars after surface flooding (Supplemental Fig. S2), a reference to the equations used during this study (Supplemental Table S1), and a reference to the transformations for statistics (Supplemental Table S2).

5.7 References

Balaine, N., T. Clough, M. Beare, S. Thomas, and E. Meenken. 2016. Relative gas diffusivity controls on soil nitrous oxide and dinitrogen fluxes and their ratios. Soil Science Society of America 80(4):529-540.

Balaine, N., T. Clough, M. Beare, S. Thomas, E. Meenken, and J. Ross. 2013. Changes in relative gas diffusivity explain soil nitrous oxide flux dynamics. Soil Science Society of America Journal 77:1496-1505.

Baral, K., A. Thomsen, J. Olesen, and S. Petersen. 2014. Controls of nitrous oxide emission after simulated cattle urine deposition. Agriculture, Ecosystems & Environment 188:103-110.

Bergstermann, A., L. Cárdenas, R. Bol, L. Gilliam, K. Goulding, A. Meijide, D. Scholefield, A. Vallejo, and R. Well. 2011. Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification. Soil Biology and Biochemistry 43:240-250.

Blakemore, L. C., P. L. Searle, and B. K. Daly. 1987. Methods for chemical analysis of soils. Pages 7-29 NZ Soil Bureau Scientific Report.

Butterbach-Bahl, K., E. Baggs, M. Dannenmann, R. Kiese, and S. Zechmeister-Boltenstern. 2013. Nitrous oxide emissions from soils: How well do we understand the processes and their controls? Philosophical Transactions of the Royal Society of London B: Biological Sciences 368.

Chapuis-Lardy, L., N. Wrage, A. Metay, J.-L. Chotte, and M. Bernoux. 2007. Soils, a sink for N_2O? A review. Global Change Biology 13:1-17.

Clough, T. J., R. R. Sherlock, K. C. Cameron, and S. F. Ledgard. 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant and Soil 178:141-152.

Davidson, E. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nature Geoscience 2:659-662.

Davidson, E., M. Keller, H. Erickson, L. Verchot, and E. Veldkamp. 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxides. BioScience 50:667-680.

de Klein, C., L. Barton, R. Sherlock, Z. Li, and R. Littlejohn. 2003. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. Australian Journal of Soil Research 41:381-399.

Dendooven, L., and J. M. Anderson. 1994. Dynamics of reduction enzymes involved in the denitrification process in pasture soil. Soil Biology and Biochemistry 26:1501-1506.

Dendooven, L., and Pemberton. 1996. Denitrification potential and reduction enzymes dynamics in a Norway spruce plantation. Soil Biology and Biochemistry 28:151-157.

Dobbie, K. E., I. P. McTaggart, and K. A. Smith. 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research 104:26891-26899.

Farquharson, R., and J. Baldock. 2008. Concepts in modelling N_2O emissions from land use. Plant and Soil 309:147-167.

Firestone, M., and E. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil. Pages 7-21 in M. O. Andreae and D. S. Schimel, editors. Exchange of Trace Gases Between Ecosystems and the Atmosphere. Wiley, New York.

Ghani, A., M. Dexter, and K. W. Perrott. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology and Biochemistry 35:1231-1243.

Goreau, T. J., W. A. Kaplan, S. C. Wofsy, M. B. McElroy, F. W. Valois, and S. W. Watson. 1980. Production of NO_2 and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Applied and Environmental Microbiology 40:526-532.

Groffman, P. M., and J. M. Tiedje. 1988. Denitrification hysteresis during wetting and drying cycles in soil. Soil Science Society of America Journal 52:1626-1629.

Guo, X., C. Drury, X. Yanga, D. Reynoldsa, and R. Fana. 2014. The Extent of soil drying and rewetting affects nitrous oxide emissions, denitrification, and nitrogen mineralization. Soil Science Society of America Journal 78:194-204.

Haynes, R. J., and P. H. Williams. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49:119-199.

Hewitt, A. E. 2010. New Zealand Soil Classification. Landcare Research Science Series No. 1. 3rd edn. Manaaki-Whenua Press, Lincoln.

Hillel, D., 1998. Environmental Soil Physics. Academic Press, San Diego, CA.

Horn, R., and A. Smucker. 2005. Structure formation and its consequences for gas and water transport in unsaturated arable and forest soils. Soil and Tillage Research 82:5-14.

IBM Corp. version 20.0. 2011. IBM SPSS Statistics [Computer software]. IBM Corp, Armonk, NY.

Kelliher, F. M., N. Cox, T. J. van der Weerden, C. A. M. de Klein, J. Luo, K. C. Cameron, H. J. Di, D. Giltrap, and G. Rys. 2014. Statistical analysis of nitrous oxide emission factors from pastoral agriculture field trials conducted in New Zealand. Environmental Pollution 186:63-66.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Kool, D. M., N. Wrage, S. Zechmeister-Boltenstern, M. Pfeffer, D. Brus, O. Oenema, and J. W. Van Groenigen. 2010. Nitrifier denitrification can be a source of N_2O from soil: a revised approach to the dual-isotope labelling method. European Journal of Soil Science 61:759-772.

Kool, D. M. M., Christoph; Wrage, Nicole; Oenema, Oene; Van Groenigen; Jan Willem 2009. Oxygen exchange between nitrogen oxides and H_2O can occur during nitrifier pathways. Soil Biology and Biochemistry 41:9.

Laughlin, R., and J. Stevens. 2002. Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. Soil Science Society of America Journal 66:1540-1548.

Linn, D. M., and J. W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal 48:1267-1272.

Luo, J., S. B. Lindsey, and S. F. Ledgard. 2008. Nitrous oxide emissions from animal urine application on a New Zealand pasture. Biology and Fertility of Soils 44:463-470.

Luxmoore, R. 1981. Micro-, meso-, and macroporosity of soil. Soil Science Society of America Journal 45:671-672.

Minitab Inc. version 17. 2010. Minitab Statistical Software [Computer software]. State College, PA.

Moldrup, P., C. Deepagoda, S. Hamamoto, T. Komatsu, K. Kawamoto, D. Rolston, and L. de Jonge. 2013. Structure-dependent Water-induced Linear Reduction model for predicting gas diffusivity and tortuosity in repacked and intact soil. Vadose Zone Journal 12:vzj2013.2001.0026.

Moldrup, P., T. Olesen, T. Komatsu, P. Schjønning, and D. E. Rolston. 2001. Tortuosity, Diffusivity, and Permeability in the Soil Liquid and Gaseous Phase. Soil Science Society of America Journal 65:613-623.

Müller, C., and R. Sherlock. 2004. Nitrous oxide emissions from temperate grassland ecosystems in the Northern and Southern hemispheres. Global Biogeochemical Cycles 18:GB1045.

Müller, C., R. J. Stevens, R. J. Laughlin, and H. J. Jäger. 2004. Microbial processes and the site of N_2O production in a temperate grassland soil. Soil Biology and Biochemistry 36:453-461.

Norton, J., and J. Stark. 2011. Regulation and measurement of nitrification in terrestrial systems. Methods in Enzymology 486:343-368.

Orwin, K. H., J. E. Bertram, T. J. Clough, L. M. Condron, R. R. Sherlock, M. O'Callaghan, J. Ray, and D. B. Baird. 2010. Impact of bovine urine deposition on soil microbial activity, biomass, and community structure. Applied Soil Ecology 44:89-100.

Owens, J., T. Clough, J. Laubach, J. Hunt, R. Venterea, and R. Phillips. 2016. Nitrous oxide fluxes, soil oxygen, and denitrification potential from urine and non-urine treated soil under different irrigation frequencies. Journal of Environmental Quality 45:1169-1177.

Parkin, T. B., R. T. Venterea, and S. K. Hargreaves. 2012. Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. Journal of Environmental Quality 41:705-715.

Petersen, S., P. Ambus, L. Elsgaard, P. Schjønning, and J. Olesen. 2013. Long-term effects of cropping system on N_2O emission potential. Soil Biology and Biochemistry 57:706-712.

Ravishankara, A. R., J. Daniel, and R. Portmann. 2009. Nitrous Oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. Science 326:123-125.

Robertson, L. A., R. Cornelisse, P. De Vos, R. Hadioetomo, and J. G. Kuenen. 1989. Aerobic denitrification in various heterotrophic nitrifiers. Antonie van Leeuwenhoek 56:289-299.

Rolston, D. E., and P. Moldrup. 2002. Gas diffusivity. Pages 1113-1139 in J. H. Dane and G. C. Topp, editors. Methods of Soil Analysis, part 4, Physical Methods. Soil Science Society of America, Madison, Wisconsin.

Schjønning, P., I. Thomsen, J. Møberg, H. de Jonge, K. Kristensen, and B. Christensen. 1999. Turnover of organic matter in differently textured soils. Geoderma 89:177-198.

Scott, H. D. (2000). Soil physics: Agricultural and environmental applications. Ames: Iowa State University Press.

Simojoki, A., and A. Jaakkola. 2000. Effect of nitrogen fertilization, cropping and irrigation on soil air composition and nitrous oxide emission in a loamy clay. European Journal of Soil Science 51:413-424.

Smith, and J. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biology and Biochemistry 11:261-267.

Smith, C. J., and W. H. Patrick. 1983. Nitrous oxide emission as affected by alternate anaerobic and aerobic conditions from soil suspensions enriched with ammonium sulfate. Soil Biology and Biochemistry 15:693-697.

Soil Survey Division Staff. 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. U. S. Department of Agriculture.

Steel, G. C., J. H. Torrie, and D. A. Dikey. 1997. Principles and procedures of statistics: A biometrical approach. McGraw-Hill.

Taghizadeh-Toosi, A., T. Clough, L. Condron, R. Sherlock, C. Anderson, and R. Craigie. 2011. Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from rumi nant urine patches. Journal of Environmental Quality 40:468-476.

Uchida, Y., T. Clough, F. Kelliher, and R. Sherlock. 2008. Effects of aggregate si ze, soil compaction, and bovine urine on N_2O emissions from a pasture soil. Soil Biology and Biochemistry 40:924-931.

Uchida, Y., Y. Wang, H. Akiyama, Y. Nakajima, and M. Hayatsu. 2014. Expression of denitrification genes in response to a waterlogging event in a Fluvisol and its relationship with large nitrous oxide pulses. FEMS Microbiology Ecology 88:407-423.

van der Weerden, T. J., T. J. Clough, and T. M. Styles. 2013. Using near-continuous measurements of N_2O emission from urine-affected soil to guide manual gas sampling regimes. New Zealand Journal of Agricultural Research 56:60-76.

Venterea, R. 2007. Nitrite-driven nitrous oxide production under aerobic soil conditions: kinetics and biochemical controls. Global Change Biology 13:1798-1809.

Venterea, R. 2010. Simplified method for quantifying theoretical underestimation of chamber-based trace gas fluxes. Journal of Environmental Quality 39:126-135.

Venterea, R. 2013. Theoretical comparison of advanced methods for calculating nitrous oxide fluxes using non-steady state chambers. Soil Science Society of America Journal 77:709-720.

Venterea, R., K. Spokas, and J. Baker. 2009. Accuracy and precision analysis of chamber-based nitrous oxide gas flux estimates. Soil Science Society of America Journal 73:1087-1093.

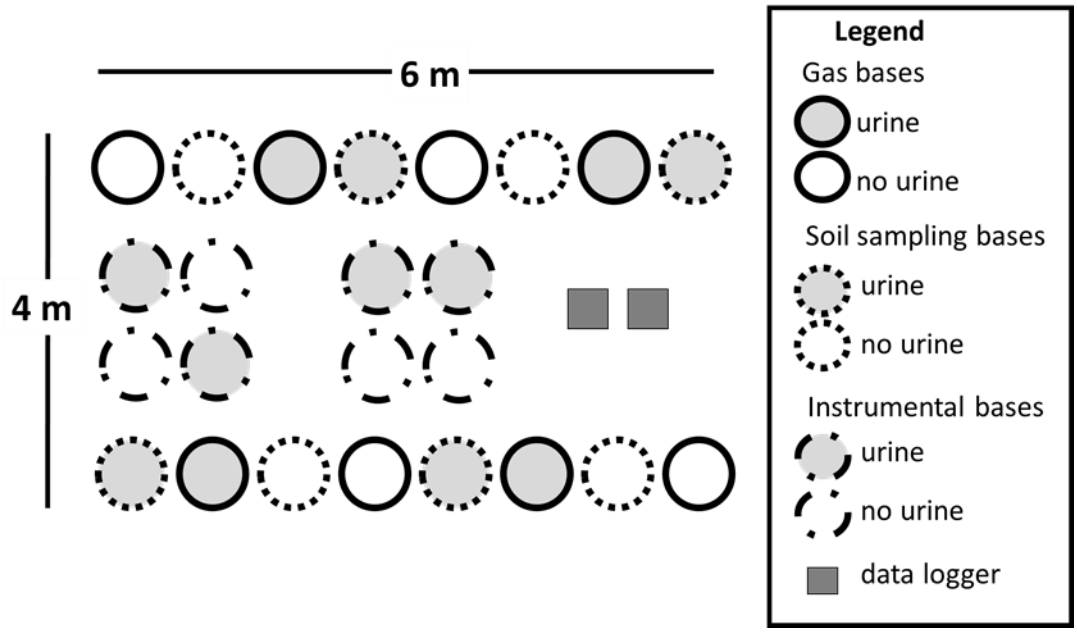
Wagner, S. W., D. C. Reicosky, and R. S. Alessi. 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal 89:279-284.

Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry 33:1723-1732.

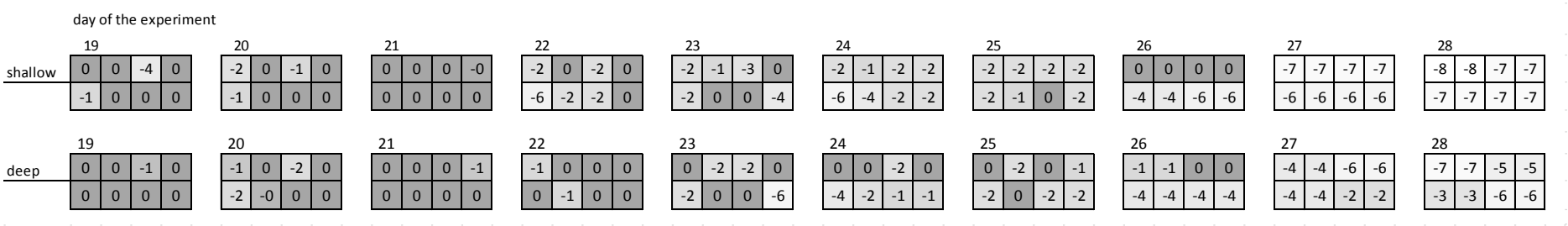
Zhu, X., M. Burger, T. Doane, and W. Horwath. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proceedings of the National Academy of Sciences 110:6328-6333.

5.8 Supplementary Data

Supplemental Fig. S1 The experimental plot showing the relative location of the collar bases for gas sampling, instrumentation, and soil sampling for the soil chemical analysis. The location of the instrumentation collars in the middle of the plot shows the location of the instrument placement relative to the gas and soil sampling collars. There were two collar bases instrumented for each treatment. Within the instrumentation collars, all of the instruments for each treatment, except the soil moisture probes, were installed within one collar, and the soil moisture produces were installed into the other.



Supplemental Fig. S2 The distribution of the soil matric potential (Ψ), which is represented by the numbers in the box, presented from day of the experiment (DOE) 19 to 28. The Ψ was measured daily from within the gas chambers from the shallow (≈20 mm depth) and deep (≈70 mm depth) measurements.



Supplemental Table S1 The equations used for soil bulk density, total porosity, gravimetric soil moisture, volumetric water content, water-filled pore space, air-filled pore space, and relative soil gas diffusivity

Variable (symbol, units	Equation	Notes
Soil bulk density (ρb, Mg m^{-3})	$\rho_b = M_s/V_s$	Ms is the mass of dry soil (Mg), and *Vs* is the volume of soil core (m^3).
Total porosity (ϕ, %)	$\Phi = \left(1 - \left(\frac{\rho_b}{\rho_d}\right)\right) * 100$	ρd is the particle density, which is assumed to be 2.65 Mg m^{-3}.
Gravimetric soil moisture (θg) (Blakemore et al. 1987)	$\theta g = M_{SW}/M_S$	M_{SW} is the mass of water and soil (Mg)
Volumetric water content (θv, m^3 m^{-3})	$\theta_v = \theta_g * (\frac{\rho_b}{\rho_d})$	
Water-filled pore space (WFPS, m^3 m^{-3}) (Linn and Doran 1984)	$WFPS = \frac{\theta v}{\Phi}$	
Air-filled pore space (ε, m^3 m^{-3})	$\varepsilon = \Phi - \theta v$	
Relative soil gas diffusivity (D_P/D_O, unit less) was modelled using the Structure-dependent Water-induced Linear Reduction (SWLR) model (Moldrup et al. 2013)	$\frac{D_P}{D_O} = \varepsilon^{[1+Cm\Phi]}\left(\frac{\varepsilon}{\Phi}\right)$	The Cm is a media complexity factor, assumed to be related to soil particle density and therefore bulk density. The Cm for soils with plants is 2.1, which was used during this study
Capillary rise equation for determining the size of soil pores which were water-filled at minimum measured soil matric potential (Ψ)	$r = \frac{0.15}{h}$	Where r is radius of pore (cm), h is the matric potential (cm H_2O) (Scott, 2000).

Supplemental Table S2 Data transformation details

Variable (units)		Transform
N_2O fluxes	Daily N_2O emissions (mg-N g^{-1} m^{-2})	Ln_{10} (N_2O)
	Cumulative N_2O emissions (mg-N g^{-1})	N/A
Soil chemical data	Nitrate (µg g^{-1} dry $soil^{-1}$)	ʎ = 0.5
	Ammonium (µg g^{-1} dry $soil^{-1}$)	Ln
	Hot water carbon (µg g^{-1} dry $soil^{-1}$)	N/A
	Cold water carbon (µg g^{-1} dry $soil^{-1}$)	ʎ = 0
	soil pH (µg g^{-1} dry $soil^{-1}$)	ʎ = 1
	Conductivity (%)	Ln
Environmental data	Soil temperature 50 mm (°C)	ʎ = 1
	Soil oxygen with diffusive head 10 mm (°C)	ʎ = 5
	Soil oxygen with diffusive head 50 mm (°C)	ʎ = 5
	Soil oxygen with diffusive head 100 mm (°C)	ʎ = 5
	Soil oxygen without diffusive head 10 mm (°C)	ʎ = 5
	Soil oxygen without diffusive head 50 mm (°C)	ʎ = 5
	Soil oxygen without diffusive head 100 mm (°C)	ʎ = 5
	Volumetric water content 50 mm	ʎ = -4
Soil physical data	Water filled pore space (m^3 m^{-3})	N/A
	Soil diffusivity (D_P/D_O)	ʎ = 0.32

Ln, natural log

Ln_{10}, log base 10

ʎ, indicates a box cox transformation was used, where the number presented is the optimal lambda

Chapter 6.

Denitrification Potential and Potential Nitrous Oxide Reductase for Denitrification Enzyme Assays Differ with Time Since a Wetting Event and with Incubation Time

6.1 Introduction

This experiment was completed to provide insight into the temporal dynamics of nitrous oxide reductase (N_2OR). There were differences in $N_2O/(N_2O+N_2)$ (called DEA-$N_2O/(DEA\text{-}N_2O+N_2)$) ratios derived from denitrification enzyme assays (DEA) observed between the two irrigation frequencies used in Experiment 1, Chapter 4, which were attributed to higher soil moisture, and therefore lower concentrations O_2 in soil micropores. While statistically different, there was little absolute difference in soil O_2 concentrations between the irrigation treatments. Was this because the average soil moisture was higher in the 3 day irrigation treatment compared to the 6 day irrigation treatment, or because there was a history of the soil being wetter for longer, leading to lower O_2 and thus priming the N_2OR pathway? Negative N_2O fluxes were observed at the end of Experiment 2, Chapter 5, following heavy irrigation. It was hypothesized that this could be attributed to priming of the N_2OR by the flooding event lowering soil O_2. This occurred prior to the irrigation induced, negative N_2O fluxes (Chapter 5). It is difficult to determine the effect that moisture history has on N_2OR function when soil moisture is continually changing. This is because soil moisture directly influences soil O_2, which is a proximal controller of N_2OR. A controlled laboratory experiment may help elucidate the temporal dynamics of N_2OR by exploring whether N_2OR responds to a history of O_2 depletion in the form of a wetting event, as was suggested in Chapter 5, and not just because the soil moisture is higher, and thus soil O_2 lower, as was suggested in Chapter 4.

High nitrous oxide (N_2O) emissions are often attributed to increased substrate availability and high soil moisture (Groffman and Tiedje 1988, Dobbie et al. 1999, Barnard et al. 2005). These factors are linked; variability in soil moisture due to alternating wet-dry cycles can increase substrate availability from biological and physical sources (Austin et al. 2004). Rewetting of dry soil can cause a "birch effect" which is a sudden change in soil osmotic pressure due to the addition of water, and can lyse cellular contents from some microbes. These highly labile cellular contents can then

serve as substrates for the enduring microbial population (Bottner 1985, Van Gestel et al. 1992). Soil aggregates may break apart following rewetting of dry soil and expose previously protected organic matter which is then available as microbial substrate (Fierer and Schimel 2002, Vor et al. 2003, Mikha et al. 2005).

Microbial functioning may also be affected by soil moisture variability, through its effect on soil O_2. Both denitrification rates, and the production of the nitrous oxide reductase enzyme (N_2OR) and thus the ratio of N_2O:N_2 emitted, are affected by O_2 concentrations. The N_2OR is inhibited by O_2, so denitrification emits N_2O disproportionality to N_2 in the presence of O_2 (Knowles 1982, Firestone and Davidson 1989, Wrage et al. 2001). While denitrification is an anaerobic process, a history of O_2 exposure can affect microbial functioning, complicating interpretation of observed relationships in soil. For example, denitrifying microbes previously exposed to anaerobic conditions may continue to denitrify and produce N_2O, in the presence of O_2 at rates of 8 to 55% of those that occurred under O_2 depleted condition (Morley et al. 2008). A history of O_2 depletion may prime the N_2OR pathway meaning that at the onset of O_2 limited conditions, N_2OR is already synthesized, or ready to be synthesized. Thus a greater pulse of N_2O will occur from previously dry soils that have been rewetted compared to rewetting soils with preceding wet conditions (Uchida et al. 2014). The lower N_2O following rewetting of soil which had previously been wet is correlated with nosZ gene expression, which is the gene that codes for N_2OR (Uchida et al. 2014). This suggests that historical effects of O_2 directly influence the ensuing microbial functioning.

Based on the concept that O_2 history affects N_2OR and therefore the ratio of gaseous end-products produced during denitrification, it is expected that after a wetting event, the ratio of $N_2O/(N_2O+N_2)$ will be low, and that it will increase with time since the wetting event in aerated soil, even if soil moisture is stable. However, soil nutrient conditions will vary after a wetting event. Deciphering the confounding effects of past and present moisture or O_2 status, substrate availably, and N_2OR production is difficult without controlled conditions.

Denitrification enzyme assays (DEA) can be used to determine the denitrification potential in soils under non-limiting conditions (Groffman et al. 1999, Drury et al. 2008), as noted in section 3.5 of this thesis. The DEA's induce anaerobic conditions to measure N_2O production under non-limiting NO_3^- and C, and compare samples with and without the presence of acetylene (C_2H_2), which inhibits N_2OR. This allows inference of N_2OR potential independent of substrate availability. This technique can aid in isolating how soil moisture history influences N_2OR over time.

Studies have used a number of variations of the DEA methodologies outlined in Smith and Tiedje (1979), and updated by Groffman et al. (1999) and Drury et al. (2008). For example, some studies add chloramphenicol to the soil. This inhibits *de novo* synthesis of N_2OR at certain concentrations, but may also inhibit existing enzymes (Pell et al. 1996). Likewise, a number of incubation times have been used during DEA's (Table 6.1). A longer incubation time during the DEA may result in greater rates of N_2OR production, and a lower ratio of DEA-N_2O/(DEA-N_2O+N_2). *De novo* synthesis of N_2OR can take between 33 and 48 h after the onset of anaerobic conditions (Firestone and Tiedje 1979, Smith and Tiedje 1979).

Table 6.1 Examples of different incubation times for denitrification enzyme assays in the literature

Incubation Time	Reference
60 minutes	Čuhel et al. (2010)
90 minutes	Smith and Parsons (1985)
3 hours	Lensi et al. (1995)
6 hours	Jha et al. (2011)
48 hours	Dendooven and Anderson (1994)
72 hours	Guo et al. (2014)
96 hours	Dendooven and Anderson (1995)
168 hours	Peterson et al. (2013)

Using DEA's can be beneficial for isolating microbial functioning by removing variability associated with nutrient limitations. However, it is wise to also consider how the methodology, specifically the anaerobic incubation time, affects the results. Thus, the objectives of this experiment are 1) quantify how DEA incubation time affects the ratio of DEA-N_2O/(DEA-N_2O+N_2), and 2) assess the influence of the time since a wetting event on denitrification potential and the DEA-N_2O/(DEA-N_2O+N_2) ratio, in order to inform the results obtained in Chapter 4 and Chapter 5.

It is hypothesized that 1) a longer DEA incubation time will result in a lower DEA-N_2O/(DEA-N_2O+N_2) ratio because the longer duration of anaerobic conditions, and non-limiting substrates, will permit more N_2OR to be synthesized, and 2) denitrification potential and the ratio of DEA-N_2O/(DEA-N_2O+N_2) will increase with time since wetting. It is expected that in drained soil, aeration will inhibit denitrification and N_2OR activity, and N_2OR will need to be resynthesized prior to N_2O reduction.

6.2 Materials and Methods

6.2.1 Soil Collection

Soil was collected from the top 100 mm of a grazed and irrigated dairy pasture located at Beacon Farm, in Te Pirita, Canterbury, New Zealand, site of Experiment 1. The soil and pasture are described in section 4.3.1 of this thesis.

6.2.2 Core Repacking

Soil was brought back to the lab and stored at 4°C overnight. Then, field moist soil was sieved to < 2 mm. Soil cores (height = 43 mm, i.d. = 37.5 mm) were repacked to a bulk density of 1.03 Mg m^{-3} using the sieved soil (section 3.2). The "wetting event" consisted of standing the repacked cores in DI water to saturate from the bottom up for 48 h. After the wetting event, soil cores were then placed on tension tables at -10 kPa to stabilize drainage at field capacity. The soil cores were held at that tension for the duration of the experiment.

6.2.3 Experiment Design and Sampling

There were two parts to the experiment; part 1 tested how the incubation time during the DEA's influenced the DEA-N_2O/(DEA-N_2O+N_2) ratio, and part 2 served to understand how time since a wetting event influenced the DEA-N_2O/(DEA-N_2O+N_2) ratio in the absence of further changes to soil moisture.

The experiment used a blocked experimental design. The repacked soil cores were randomly placed on one of four tension tables during the experiment. Time since the wetting event was considered the treatment. Four soil cores (one from each block) were destructively sampled nine times throughout the experiment (Figure 6.1). Day of experiment (DOE) 0 represented the first destructive sampling of soil cores which occurred immediately after the 48 h wetting event (Figure 6.1).

6.2.3.1 Denitrification Enzyme Assay to Determine Denitrification Potential

Soil from the destructive samples were used for determining denitrification potential on DOE 0, 4, 7, 11, 14, 18, 21, 28 and 42 (Figure 6.1, part 2). Conditions for denitrification are optimal during DEA's, that is, there is strict anaerobiosis, no limitation in NO_3^- availability, and the incubations are

run as slurries to eliminate spatial factors. Denitrification enzyme assays, from which the denitrification potential and DEA-N_2O/(DEA-N_2O+N_2) ratio were derived, used the acetylene inhibition technique (Drury et al. 2008), as detailed in section 3.5 of this thesis. For the DEA, soils were incubated for a total of 48 h, with DEA-N_2O concentrations measured every 4 h (Figure 6.1, part 1).

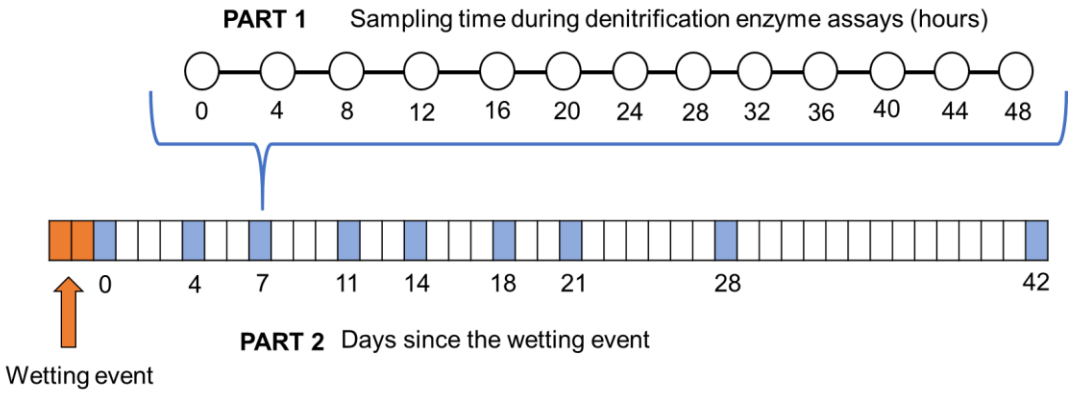


Figure 6.1 Part 1, the frequency of N_2O sampling (in hours) during the 48 hour denitrification enzyme assay (DEA), and Part 2, the timing of the destructive soil cores sampling according to treatment (days since the wetting event), where the blue boxes represent days on which DEA's commenced.

6.2.4 Ancillary Soil Environmental Data

Soils from the destructive samples were also used for determining soil pH and gravimetric soil moisture (θg) on DOE 0, 4, 7, 11, 14, 18, 21, 28, and 42. On DOE 0, 7, 14, 21, 28, and 42, inorganic N (NO_3^--N, and ammonium, NH_4^+-N) and organic C (cold water carbon, CWC, and hot water carbon, HWC) were also measured (see section 3.3).

Using θg and soil bulk density, volumetric water content (θv) and water-filled pore space (WFPS) were calculated (section 3.1). The relative soil gas diffusivity (D_P/D_O) was modelled using a media complexity factor representing local-scale soil heterogeneity of 1, which has been found to be a good fit for repacked soil cores (Moldrup et al. 2013a), as detailed in section 3.1.

6.2.5 Data Presentation and Analyses

Data analyses were completed using Minitab (Minitab Inc. version 17 2010) with parametric statistics. The normality of the data distribution for each variable was assessed using the Shapiro

Wilk test ($P < 0.05$) and each variable was also assessed for Skewness and Kurtosis. Non-normally distributed variables were transformed using box-cox transforms (Supplementary Table S1).

6.2.5.1 Part 1 - Effect of Incubation Time on DEA-N_2O/(DEA-N_2O+N_2)

To determine if DEA-N_2O+N_2, DEA-N_2O and DEA-N_2O/(DEA-N_2O+N_2) varied with incubation time, the data from each sampling time since the wetting event were pooled and are presented. The rates of change in DEA-N_2O and DEA-N_2O+N_2 with incubation time were determined from the slope of a simple linear regression, with incubation time (h) as the independent variable, and either DEA-N_2O or DEA-N_2O+N_2 as the dependent variable.

6.2.5.2 Part 2 - Effects of Time Since the Wetting Event on DEA-N_2O/(DEA-N_2O+N_2)

The effect of the time since a wetting event on DEA-N_2O/(DEA-N_2O+N_2) was assessed using the DEA-N_2O accumulation over the 48 hour incubation time from the without C_2H_2 (DEA-N_2O) and the with C_2H_2 (DEA-N_2O+N_2) samples. Significant N_2OR activity was determined by comparing DEA-N_2O+N_2 and DEA-N_2O at each sampling time using the least significant difference (LSD), which was calculated at each time point. The LSD was calculated using the pooled standard error of the difference between the DEA-N_2O and DEA-N_2O+N_2 at each sampling point. To get error bars at each point, this pooled standard error was multiplied by a t-critical value (4.303) for a probably (P) of 0.05. This calculation was used because it allows for determination of the differences between means - like a t-statistic - but with a more comprehensive estimate of the error in the data because it uses the pooled error variance for making mean comparisons. Simple linear regression was used to test for relationships between DEA-N_2O+N_2, DEA-N_2O, and DEA-N_2O/(DEA-N_2O+N_2) which were the dependent variables, and the soil environmental factors (θg, θv, WFPS, D_P/D_O, pH, NO_3^-, NH_4^+, CWC, and HWC), which were the independent variables.

6.3 Results

6.3.1 Part 1 - Effect of Incubation Time on Denitrification Potential and Potential DEA-N_2O/(DEA-N_2O+N_2) Ratio

The lowest DEA-N_2O and DEA-N_2O+N_2 fluxes were observed after 4 h of incubation, the shortest incubation time (Figure 6.2). Both DEA-N_2O+N_2 and DEA-N_2O increased with longer incubation times (Figure 6.2) and maximum DEA-N_2O and DEA-N_2O+N_2 fluxes were observed at 48 h. The rate

of increase with incubation time, as shown by the slope of a linear regression, was greater with the DEA-N_2O+N_2 compared to the DEA-N_2O (Figure 6.2).

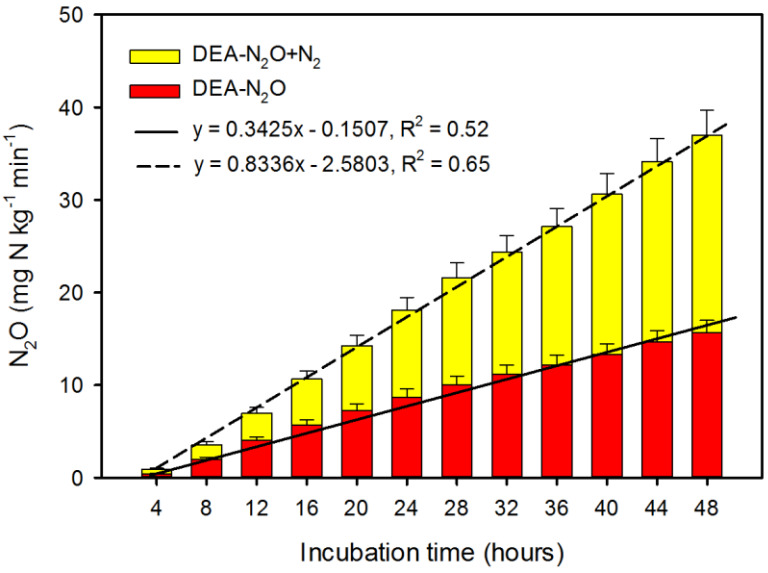


Figure 6.2 The average potential DEA-N_2O and potential DEA-N_2O+N_2 (±SEM) for each incubation time, for all times since the wetting event

Incubation time influenced the average ratio of DEA-N_2O/(DEA-N_2O+N_2). The ratio increased from the 4 h incubation time, reaching a maximum at 12 h, before gradually decreasing to a minimum at 48 h (Figure 6.3). The ratio of DEA-N_2O/(DEA-N_2O+N_2) is generally higher with shorter incubation time (Figure 6.4) and more variability in the ratios was observed with time since a wetting event with a shorter incubation time (Figure 6.4). The ratio remained highest at DOE 11 when comparing all incubation times (Figure 6.4).

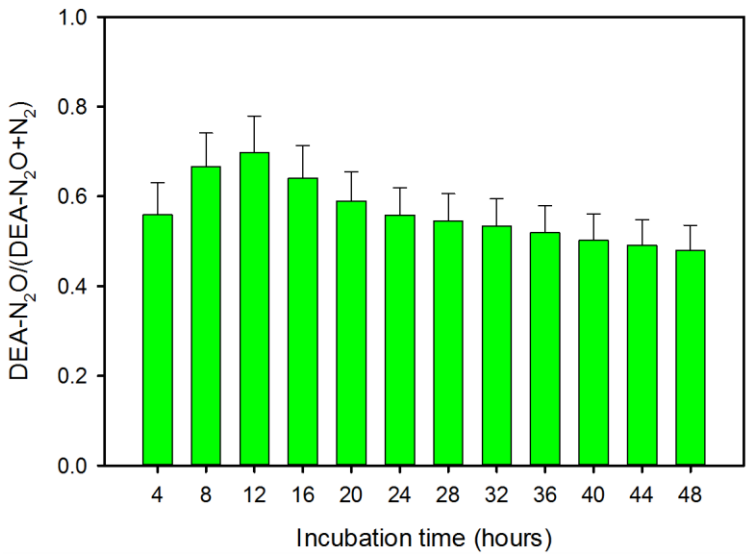


Figure 6.3 The average potential ratio of DEA-N_2O/(DEA-N_2O+N_2) (±SEM) for each incubation time, for all times since the wetting event

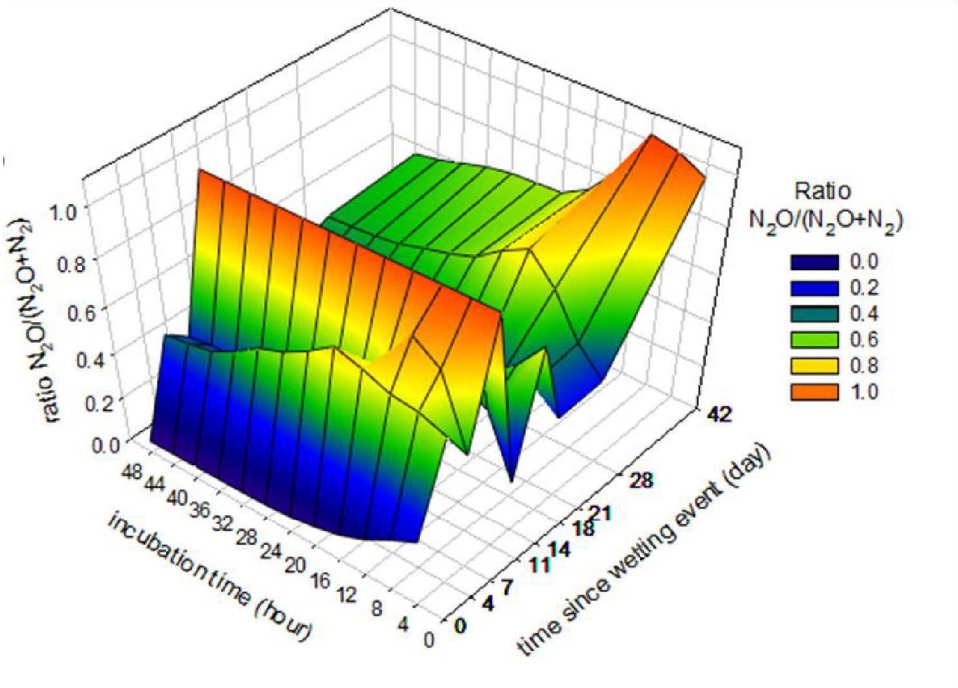


Figure 6.4 A 3D representation of the time since wetting treatment and the incubation time effects on the average ratio of DEA-N_2O/(DEA-N_2O+N_2).

6.3.2 Part 2 - Changes in Denitrification Potential and Potential DEA-N_2O/(DEA-N_2O+N_2) Ratio After a Wetting Event

This section used data derived from after 48 h of the DEA incubations to be consistent with the methods used in Experiment 1, Chapter 4. After the wetting event, drainage caused WFPS to decrease (Figure 6.5 a) and D_P/D_O to increase (Figure 6.5 b), with both remaining relatively stable from DOE 4 onwards. Both NH_4^+-N (Figure 6.5 c) and CWC (Figure 6.5 e) decreased rapidly after the wetting event, while NO_3^--N (Figure 6.5 d) gradually increased. The HWC increased after the wetting event, peaking on DOE 7, before decreasing thereafter (Figure 6.5 f). Soil pH decreased from 5.6 after the wetting event to 5.2 on DOE 7. It increased again on DOE 11 and 14 to ~5.4 before gradually decreasing again to ~5.2, remaining steady for the rest of the experiment (Figure 6.5 g).

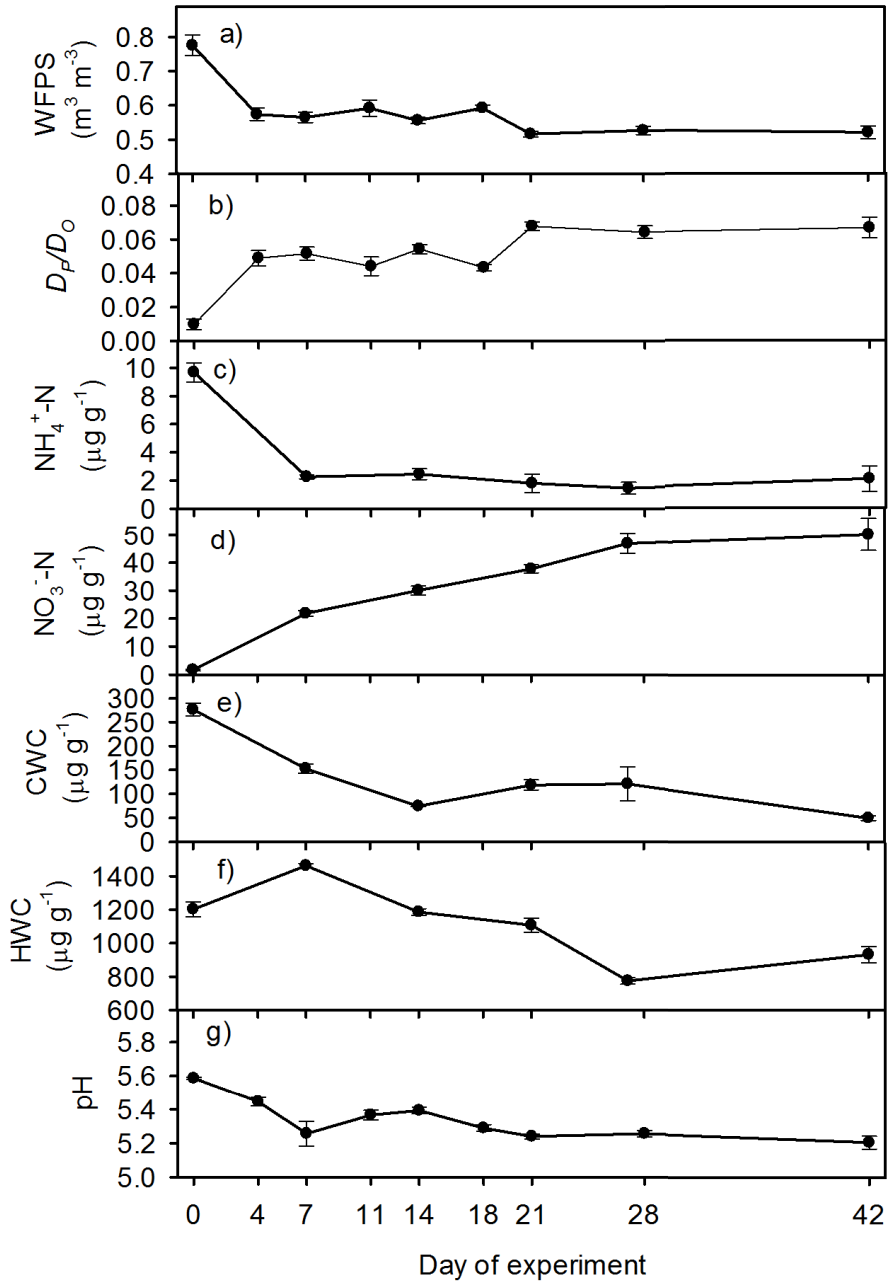


Figure 6.5 Changes in mean (±SEM, n = 4) a) water-filled pore space, b)relative soil gas diffusivity, c) ammonium-N, d) nitrate-N, e) cold water carbon, f) hot water carbon, and g) soil pH, over time.

Following the wetting event, both DEA-N_2O+N_2 and DEA-N_2O increased, peaking on DOE 4 (Figure 6.6). The DEA-N_2O flux was steady between DOE 7 and 21 before decreasing slightly thereafter (Figure 6.6). The DEA-N_2O+N_2 also decreased after DOE 4, reaching a minimum on DOE 11. It increased again, peaking a second time on DOE 18 before decreasing (Figure 6.6). The LSD results show DEA-N_2O+N_2 was higher than DEA-N_2O on DOE 0, 4, 7, 14, and 18 (Figure 6.6).

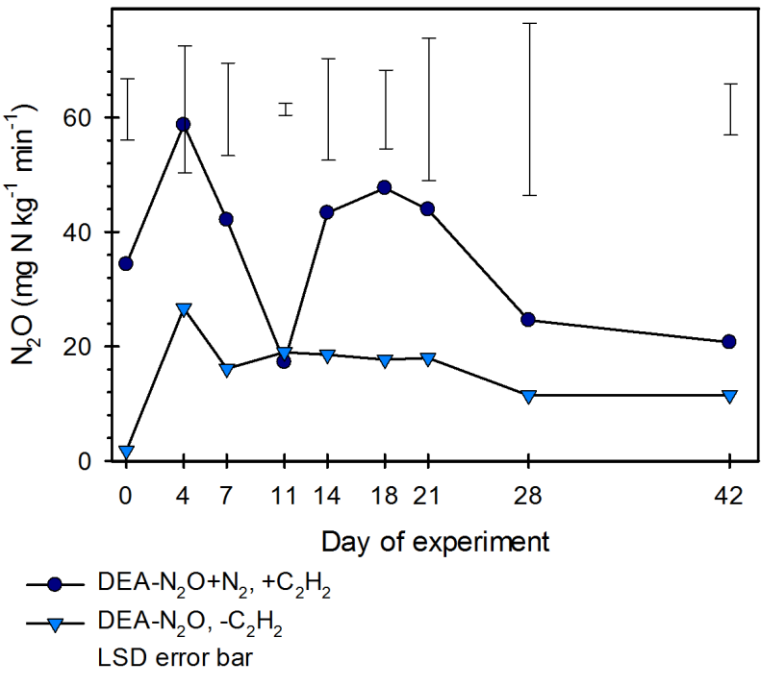


Figure 6.6 Mean nitrous oxide fluxes from the "with acetylene" treatment representing potential N_2O+N_2 fluxes and the nitrous oxide fluxes from the "without acetylene" treatment, representing potential N_2O fluxes over time, with the error bars representing the least significant difference.

After the wetting event, the DEA-N_2O/(DEA-N_2O+N_2) ratio increased from 0.05 on DOE 0, peaking on DOE 11 at 1, before declining to 0.43 on DOE 14. After DOE 14, the ratio gradually increased to ≈0.56 by DOE 42 (Figure 6.7).

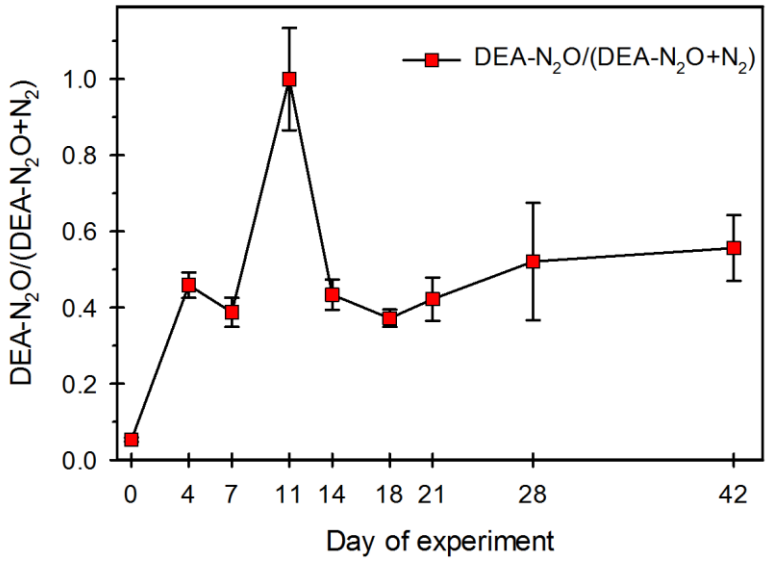


Figure 6.7 Mean ratio of potential N_2O and potential N_2O+N_2 from the denitrification enzyme, over the course of the experiment (n = 3, ± SEM)

The DEA-N_2O+N_2 flux was negatively related to NO_3^--N and positively related to HWC (Figure 6.6). More soil environmental factors were individually related to DEA-N_2O; soil pH and NO_3^--N were positively related to DEA-N_2O, and NH_4^+-N and CWC were negatively related to DEA-N_2O (Figure 6.6). The DEA-N_2O/(DEA-N_2O+N_2) ratio was positively related to NO_3^--N and negatively CWC (Figure 6.6).

Table 6.2 Linear regression analysis for assessing the influence of soil chemical and hydrological data on DEA-N_2O+N_2, DEA-N_2O, and the ratio of DEA-N_2O/(DEA-N_2O+N_2). The "+" or "-" sign in front of the r^2 value represents whether the relationship between the variables are positive or negative, respectively.

Variable	DEA-N_2O+N_2 (mg N kg^{-1} min^{-1})		DEA-N_2O (mg N kg^{-1} min^{-1})		Ratio DEA-N_2O/(DEA-N_2O+N_2)	
	P-value	r^2	P-value	r^2	P-value	r^2
pH	0.438	+ 0.02	0.023	+ 0.28	0.143	- 0.84
NH_4^+-N (µg g^{-1})	0.767	- 0.06	0.000	- 0.55	0.000	- 0.57
NO_3^--N (µg g^{-1})	0.051	- 0.22	0.056	+ 0.21	0.000	+ 0.66
CWC (µg g^{-1})	0.104	+ 0.16	0.035	- 0.25	0.000	- 0.60
HWC(µg g^{-1})	0.003	+ 0.43	0.067	+ 0.19	0.696	- 0.10
WFPS	0.803	+ 0.03	0.066	- 0.13	0.083	- 0.12
D_P/D_O	0.839	- 0.02	0.053	+ 0.14	0.075	+ 0.12

6.4 Discussion

It was expected that both a longer incubation time and a longer time after a wetting event would result in lower DEA-N_2O/(DEA-N_2O+N_2) ratios. The results from this study showed longer incubation times generally equated to greater denitrification potential and lower DEA-N_2O/(DEA-N_2O+N_2) ratios, supporting the original hypothesis. The effect of the soil moisture history was less clear; the DEA-N_2O/(DEA-N_2O+N_2) ratio was variable shortly after the wetting event, but generally increased with time since a wetting event, over the course of the experiment. These results can be attributed to more NO_3^-, less C, and lower pH.

6.4.1 Part 1 - Effects of Incubation Time on Denitrification Potential and $N_2O/(N_2O+N_2)$

Nitrous oxide reductase enzymes can take up to 48 hours to synthesize (Firestone and Tiedje 1979), which explains why the DEA-N_2O/(DEA-N_2O+N_2) increases with incubation time. The high DEA-N_2O/(DEA-N_2O+N_2) ratio at h 12 of the incubations (Figure 6.3) suggests pre-existing or already synthesized N_2OR was responsible for the low ratio of DEA-N_2O/(DEA-N_2O+N_2) at h 4 and 8 of the incubations. Pre-existing N_2OR enzymes may be de-repressed and used shortly after the induction of O_2 depleted conditions (Firestone and Tiedje 1979, Smith and Parsons 1985), such as during the DEA incubations. This 12 h mark has been noted in other studies. It has been found that prior to 12 h of anaerobiosis, N_2O production increases, but after 12 h of anaerobiosis, the net rate of N_2O production decreases as N_2O production is outpaced by reduction (Firestone and Tiedje 1979). Despite the well-drained status of the cores while on the tension tables after the wetting event, not all soil pores would have drained, leaving microsites for denitrification to occur (Müller et al. 2004). The contribution of microsites to denitrification explains the evidence of potential N_2OR before hour 12 of the DEA incubations. After the 12 h mark during the incubations in this current study, newly synthesized N_2OR was likely responsible for the lower DEA-N_2O/(DEA-N_2O+N_2).

Non-limiting conditions for denitrification imposed by the DEA explain the continued gas production with longer incubation times (Figure 6.2). There is some conflict in the literature about why this occurs. It could be due to maximized N_2OR production from the existing microbial population (Firestone and Tiedje 1979), or an increase in the size of the microbial population size, indicated by a logarithmic increase in gas production (Smith and Tiedje 1979). An increase in

microbial population size cannot be excluded in this study without a direct measure of microbial population. However, there was no logarithmic increase in gas production observed during the DEA incubations in this study (Supplementary Figure S1) suggesting little growth of the microbial communities during the 48 hour incubation period.

6.4.2 Part 2 - Effects of Time Since a Wetting Event on Denitrification Potential and DEA-N_2O/(DEA-N_2O+N_2)

Few studies have examined temporal effects of N_2OR associated with soil moisture history. The effect of time since the wetting event on DEA-N_2O/(DEA-N_2O+N_2) was not a consistent linear increase (Figure 6.6), nor did the DEA-N_2O+N_2 or DEA-N_2O rates decrease linearly with time since the wetting event (Figure 6.7). The DEA-N_2O+N_2 flux was more variable with time since wetting when compared to the DEA-N_2O flux, which is reflected in the DEA-N_2O/(DEA-N_2O+N_2) ratio. This suggests N_2OR varied with time since a wetting event, despite constant soil moisture after the wetting event (Figure 6.7). The low DEA-N_2O/(DEA-N_2O+N_2) ratio immediately after the wetting event can be attributed to complete denitrification (reduction of N_2O to N_2) induced by O_2 limitation associated with the wetting event promoting N_2OR (Balaine et al. 2016). With soil drainage on the tension tables at -10 kPa, the increase in the DEA-N_2O/(DEA-N_2O+N_2) ratio with time since the wetting event (Figure 6.7) suggests N_2OR potential decreases the longer the soil is aerated, supporting the second hypothesis of this study.

The variability in DEA-N_2O/(DEA-N_2O+N_2) with time since wetting cannot be attributed to soil moisture variability, since soil moisture was stable, and controlled with the use of tension tables. Nitrous oxide reductase, while highly sensitive to O_2 (Knowles 1982, Wrage et al. 2001), has been found to persist in the presence of O_2, in dry soil that has been rewet (Smith and Parsons 1985). It is clear from the results in this experiment that N_2OR activity changes despite stability in soil moisture. Nitrous oxide reductase activity has been found to be influenced by soil moisture history (Uchida et al. 2014) but the current study did not measure N_2OR from microbial activity directly. However, the methods employed allow for isolation of any affect changes in soil moisture may have had, from the effects of changes in soil chemistry, on potential N_2OR. It is clear from these results that N_2OR activity changes despite the stability in soil moisture and D_P/D_O. This suggests that there are either residual biological effects from the antecedent moisture conditions, or the N_2OR is affected by the other, ancillary environmental conditions like pH and NO_3^- availability prior to the DEA being undertaken.

The increase in NO_3^--N and decrease in NH_4^+-N (Figure 6.5) in the soil cores with time since wetting is indicative of nitrification (Firestone and Davidson 1989). At -10 kPa, the soil cores on the tension tables were well aerated after the wetting event (Figure 6.5) thus nitrification, an aerobic process (Norton and Stark 2011, Ward 2013), likely occurred. This is supported by D_P/D_O values being above 0.02 after DOE 0, which is indicative of aeration (Stepniewski 1981).

The abrupt increase in the DEA-N_2O/(DEA-N_2O+N_2) ratio on DOE 11 (Figure 6.7) may be partially attributed to the changes in pH. The changes in pH may be induced by nitrification which could subsequently affect N_2OR. High soil pH promotes an increase in denitrification rates. A decrease in soil pH may explain the decrease in DEA-N_2O+N_2 and DEA-N_2O between DOE 7 and 11, leading to a higher DEA-N_2O/(DEA-N_2O+N_2) ratios from denitrification (Knowles 1982, Wallenstein et al. 2006, Samad et al. 2016). However, while pH varies with time since the wetting event (Figure 6.5), it fails to match up directly with the anomaly observed on DOE 11.

The benefit of adding NO_3^- and C to the soil during the enzyme assays is that it allows for isolation of the effect of time since wetting on N_2OR, since both NO_3^- and C concentrations limitations affect our ability to measure the DEA-N_2O/(DEA-N_2O+N_2) ratio. Nitrate can be used as a terminal electron acceptor over N_2O during denitrification (Knowles 1982) so the presence of NO_3^- inhibits N_2O reduction to N_2. However, the presence of NO_3^- ensures denitrification occurs, which is pertinent to the denitrification potential measurements. The DEA's supply 50 µg g^{-1} of NO_3^-, but total NO_3^- varied over time due to changes in indigenous NO_3^- concentrations within the soil cores (increasing from 1.68 to 50.26 µg g^{-1}). More relevantly, the presence of N oxides in soil might also influence N_2OR synthesis. It has been found that the amount of N_2OR in cells grown on NO_3^- media was high, although enzyme activity was low. Cells grown in the absence of NO_3^- had about 10% the amount of enzyme of cells grown in the presence of NO_3^-, yet specific activity of the enzymes in the former cells was 3 to 4 times greater (Zumft et al. 1985). This perhaps suggests that as NO_3^- concentrations increase in soil, more N_2OR was synthesized in the cell, but that the absolute amount of NO_3^- in the soil during the DEA incubation, which was 50 µg g^{-1} supplied in the DEA slurry, along with the NO_3^- which was in the soil (increasing from 1.68 to ~50.26 µg g^{-1} by the end of the experiment on DOE 42), contributed to the increase in the DEA-N_2O/(DEA-N_2O+N_2). Carbon availability results in higher denitrification rates (Smith and Tiedje 1979) and possibly N_2OR production/activity, through lowering O_2 by stimulation of heterotrophic respiration (Morley and Baggs 2010). Heterotrophic denitrifiers require a source of C, so indigenous soil C availability, as well as the ratio of NO_3^- to C, can influence the ratio of gaseous end-products (Firestone and

Davidson 1989, Holtan-Hartwig et al. 2000, Azam et al. 2002). The negative relationship to C may have resulted in more C respiration, and consequently lower soil O_2, which would favour N_2OR (Morley and Baggs 2010). Thus the positive relationship between DEA-N_2O/(DEA-N_2O+N_2) and NO_3^--N, and the negative relationship between CWC and DEA-N_2O/(DEA-N_2O+N_2) (Table 6.2), are in line with previous findings.

This study informs the results obtained from Chapter 4. These results suggest that the higher DEA-N_2O/(DEA-N_2O+N_2) observed under the 3 day irrigation frequency compared to the 6 day irrigation frequency (Figure 4.2). This was probably not only due to the comparatively higher soil moisture under the more frequent irrigation regime (Figure 4.1), but could also due to the sustained higher N_2OR potential after the irrigation event because there is more N_2OR closer to a wetting event.

The results from this experiment also help explain some of the results from Chapter 5. During surface flooding, N_2O production was high, but after this, following heavy irrigation, N_2O fluxes increased temporarily and were followed by instances of N_2O uptake. Why did one instance of moisture increase result in high N_2O fluxes while the other resulted in N_2O uptake? This can be attributed to the effects of moisture history on N_2OR. The results from the current study suggest that the surface flooding induced N_2O fluxes, which preceded the irrigation induced N_2O uptake, were likely due to the surface flooding event reducing soil O_2, and this O_2 reduction primed the N_2OR pathway. This priming of the N_2OR pathway meant that when soil moisture increased again and soil O_2 decreased with the heavy irrigation, N_2O uptake outpaced N_2O production, and negative N_2O fluxes occurred. Nitrous oxide reductase was sustained following the flooding, and quickly de-repressed with irrigations events, leading to uptake of N_2O. Future microbial studies are required to look at this effect on a molecular level.

6.5 Conclusions

This study demonstrates that incubation time will influence de nitrification potential results. It is likely pre-existing N_2OR is responsible for the lower ratio of DEA-N_2O/(DEA-N_2O+N_2) observed within the first 12 h of the incubation. After the 12 h mark, the lower ratio of DEA-N_2O/(DEA-N_2O+N_2) was likely newly synthesized N_2OR.

After a wetting event, there is evidence of high potential N_2OR, as shown by the ratio of DEA-N_2O/(DEA-N_2O+N_2). Once soil is drained and aerated, the N_2OR potential decreases over time in the absence of soil moisture variability. This study highlights the importance of soil nutrient

concentrations on the DEA-N_2O/(DEA-N_2O+N_2) ratio with greater NO_3^--N resulting in higher DEA-N_2O/(DEA-N_2O+N_2) ratios. The scale of NO_3^- in the soil during the current experiment is relatively low compared to that found in a urine patch. Given that NO_3^- concentration affects N_2OR kinetics, future work should focus on the effects of NO_3^- levels in combination with different permutations of environmental factors, such as pH, on N_2O:N_2 ratios.

This experiment does not include plants, but the rhizosphere will also influence the soil nutrient status. The influence of plants on DEA-N_2O/(DEA-N_2O+N_2) ratios should be explored to help translate the laboratory results to field scenarios.

Supplementary Data

The supplementary data shows the transformations used for the statistics (Supplementary Table S1), the kinetics of the N_2O fluxes measured for each incubation (Supplementary Figure S1), the changes in fluxes from the DEA-N_2O+N_2 (+C_2H_2) and DEA-N_2O (-C_2H_2) from each incubations (Supplementary Figure S2), and scatter plots of DEA-N_2O+N_2, DEA-N_2O, and DEA-N_2O/(DEA-N_2O+N_2) against soil environmental variables (Supplementary Figure S3).

Acknowledgements

This research was undertaken with CRI Core Funding to Landcare Research from New Zealand's Ministry of Business, Innovation and Employment. Many thanks to Neil Smith for technical assistance, and to the Centre for Environment and Soil Research at Lincoln University for the loaned equipment.

6.6 References

Austin, A., L. Yahdjian, J. Stark, J. Belnap, A. Porporato, U. Norton, D. Ravetta, and S. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221-235.

Azam, F., C. Müller, A. Weiske, G. Benckiser, and J. Ottow. 2002. Nitrification and denitrification as sources of atmospheric nitrous oxide – role of oxidizable carbon and applied nitrogen. Biology and Fertility of Soils 35:54-61.

Balaine, N., T. Clough, M. Beare, S. Thomas, and E. Meenken. 2016. Relative gas diffusivity controls on soil nitrous oxide and dinitrogen fluxes and their ratios. Soil Science Society of America. DOI: 10.2136/sssaj2015.09.0350

Barnard, R., P. Leadley, and B. Hungate. 2005. Global change, nitrification, and denitrification: A review. Global Biogeochemical Cycles 19:GB1007.

Bottner, P. 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C- and ^{15}N-labelled plant material. Soil Biology and Biochemistry 17:329-337.

Čuhel, J., M. Šimek, R. Laughlin, D. Bru, D. Chèneby, C. Watson, and L. Philippot. 2010. Insights into the Effect of Soil pH on N_2O and N_2 Emissions and Denitrifier Community Size and Activity. Applied and Environmental Microbiology 76:1870-1878.

Dendooven, L., and J. M. Anderson. 1994. Dynamics of reduction enzymes involved in the denitrification process in pasture soil. Soil Biology and Biochemistry 26:1501-1506.

Dendooven, L., and J. M. Anderson. 1995. Maintenance of denitrification potential in pasture soil following anaerobic events. Soil Biology and Biochemistry 27:1251-1260.

Dobbie, K. E., I. P. McTaggart, and K. A. Smith. 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research 104:26891-26899.

Drury, C. F., D. D. Myrold, E. G. Beauchamp, and W. D. Reynolds. 2008. Denitrificaiton techniques for soil. Pages 471-493 in M. R. Carter and E. G. Gregorich, editors. Soil Sampling and Methods of Analysis. CRC Press, Boca Raton, FL, USA.

Fierer, N., and J. Schimel. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34:777-787.

Firestone, M., and E. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil.in M. O. Andreae and D. S. Schimel, editors. Exchange of Trace Gases Between Ecosystems and the Atmosphere. Wiley, New York.

Firestone, M. K., and J. M. Tiedje. 1979. Temporal change in nitrous oxide and dinitrogen from denitrification following onset of anaerobiosis. Applied and Environmental Microbiology 38:673-679.

Groffman, P. M., E. A. Holland, D. D. Myrold, G. P. Robertson, and X. Zou. 1999. Denitrification. Pages 272-290 in G. P. Robertson, C. S. Bledsoe, D. C. Coleman, and P. Sollins, editors. Standard Soil Methods for Long-Term Ecological Research. Oxford Press, New York.

Groffman, P. M., and J. M. Tiedje. 1988. Denitrification hysteresis during wetting and drying cycles in soil. Soil Science Society of America Journal 52:1626-1629.

Guo, X., C. Drury, X. Yanga, D. Reynoldsa, and R. Fana. 2014. The Extent of Soil Drying and Rewetting Affects Nitrous Oxide Emissions, Denitrification, and Nitrogen Mineralization. Soil Science Society of America 78:194-204.

Holtan-Hartwig, L., P. Dorsch, and L. R. Bakken. 2000. Comparison of denitrifying communities in organic soils: Kinetics of NO_3^- and N_2O reduction. Soil Biology and Biochemistry 32:833-843.

Jha, N., S. Saggar, R. W. Tillman, and D. Giltrap. 2011. Preliminary studies to measure denitrification enzyme activity and denitrification rate in New Zealand pasture soils. Palmerston North, New Zealand.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Lensi, R., A. Clays-Josserand, and L. Jocteur Monrozier. 1995. Denitrifiers and denitrifying activity in size fractions of a mollisol under permanent pasture and continuous cultivation. Soil Biology and Biochemistry 27:61-69.

Mikha, M., C. Rice, and G. Milliken. 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biology and Biochemistry 37:339-347.

Minitab Inc. version 17. 2010. Minitab Statistical Software [Computer software]. State College, PA.

Moldrup, P., T. K. K. Chamindu Deepagoda, S. Hamamoto, T. Komatsu, K. Kawamoto, D. E. Rolston, and L. Wollesen de Jonge. 2013. Structure-Dependent Water-Induced Linear Reduction Model for Predicting Gas Diffusivity and Tortuosity in Repacked and Intact Soil. Vadose Zone Journal 12:2-11.

Morley, N., E. Baggs, P. Dörsch, and L. Bakken. 2008. Production of NO, N_2O and N_2 by extracted soil bacteria, regulation by NO_2^- and O_2 concentrations. FEMS Microbiology Ecology 65:102-112.

Morley, N., and E. M. Baggs. 2010. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biology and Biochemistry 42:1864-1871.

Müller, C., R. J. Stevens, R. J. Laughlin, and H. J. Jäger. 2004. Microbial processes and the site of N_2O production in a temperate grassland soil. Soil Biology and Biochemistry 36:453-461.

Norton, J., and J. Stark. 2011. Regulation and measurement of nitrification in terrestrial systems. Methods in Enzymology 486:343-368.

Pell, M., B. Stenberg, J. Stenström, and L. Torstensson. 1996. Potential denitrification activity assay in soil—With or without chloramphenicol? Soil Biology and Biochemistry 28:393-398.

Peterson, M. E., D. Curtin, S. Thomas, T. J. Clough, and E. D. Meenken. 2013. Denitrification in vadose zone material amended with dissolved organic matter from topsoil and subsoil. Soil Biology and Biochemistry 61:96-104.

Samad, M., L. Bakken, S. Nadeem, T. Clough, C. de Klein, K. Richards, G. Lanigan, and S. Morales. 2016. High-Resolution Denitrification Kinetics in Pasture Soils Link N_2O Emissions to pH, and Denitrification to C Mineralization. PLoS ONE 11:e0151713.

Smith, and J. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biology and Biochemistry 11:261-267.

Smith, M. S., and L. L. Parsons. 1985. Persistence of denitrifying enzyme activity in dried soils. Applied and Environmental Microbiology 49:316-320.

Stepniewski, W. 1981. Oxygen diffusion and the strength as related to soil compaction. II Oxygen diffusion coefficient. Polish Journal of Soil Science 14:3-13.

Uchida, Y., Y. Wang, H. Akiyama, Y. Nakajima, and M. Hayatsu. 2014. Expression of denitrification genes in response to a waterlogging event in a Fluvisol and its relationship with large nitrous oxide pulses. FEMS Microbiology Ecology 88:407-423.

Van Gestel, M., J. N. Ladd, and M. Amato. 1992. Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. Soil Biology and Biochemistry 24:103-111.

Vor, T., J. Dyckmans, N. Loftfield, F. Beese, and H. Flessa. 2003. Aeration effects on CO_2, N_2O, and CH4 emission and leachate composition of a forest soil. Z. Pflanzenernähr. Bodenk. 166:39-45.

Wallenstein, M., D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications 16:2143-2152.

Ward, B. B. 2013. Nitrification. Reference Module in Earth Systems and Environmental Sciences. Elsevier.

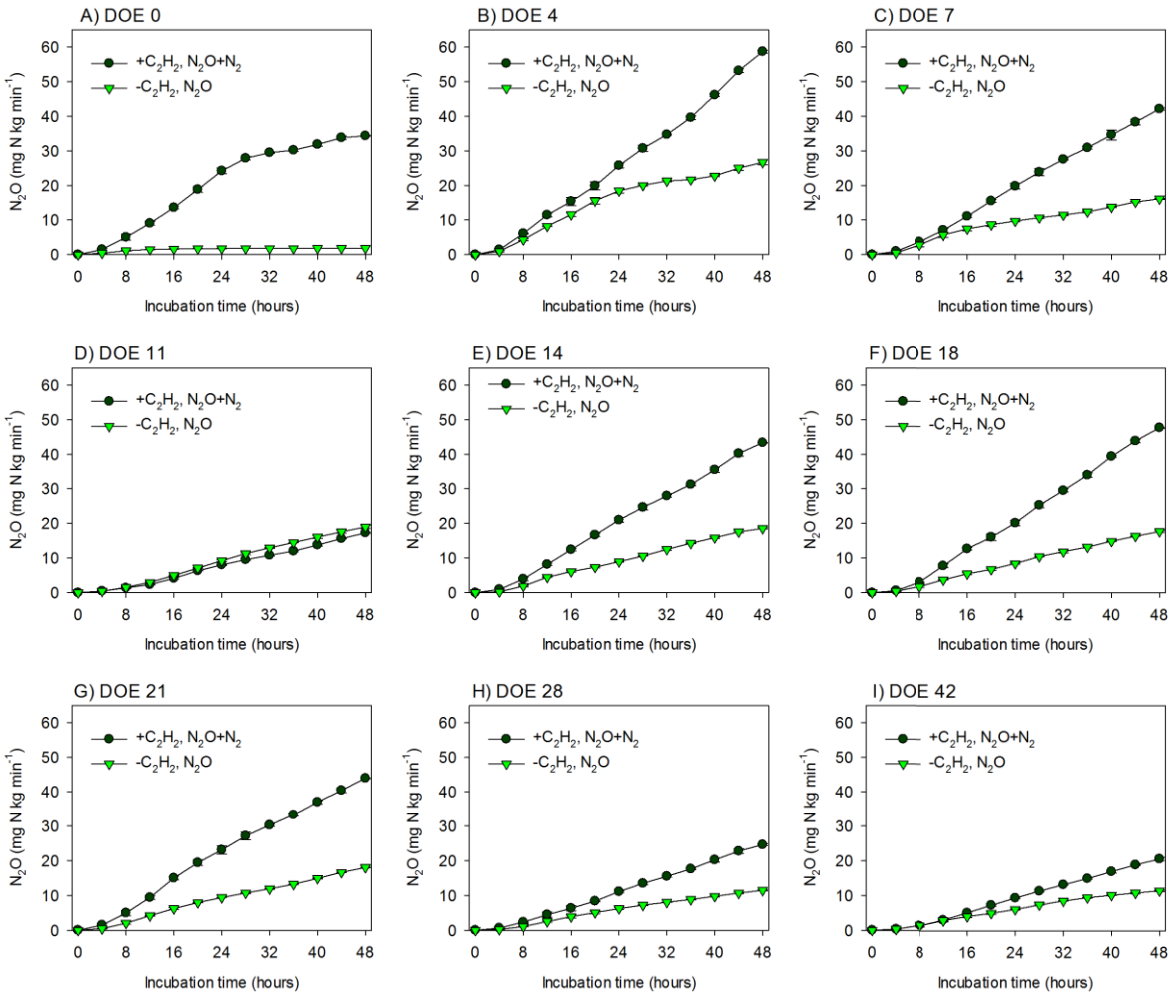
Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry 33:1723-1732.

Zumft, W. G., K. Döhler, and H. Körner. 1985. Isolation and characterization of transposon Tn5-induced mutants of Pseudomonas perfectomarina defective in nitrous oxide respiration. Journal of Bacteriology 163:918-924.

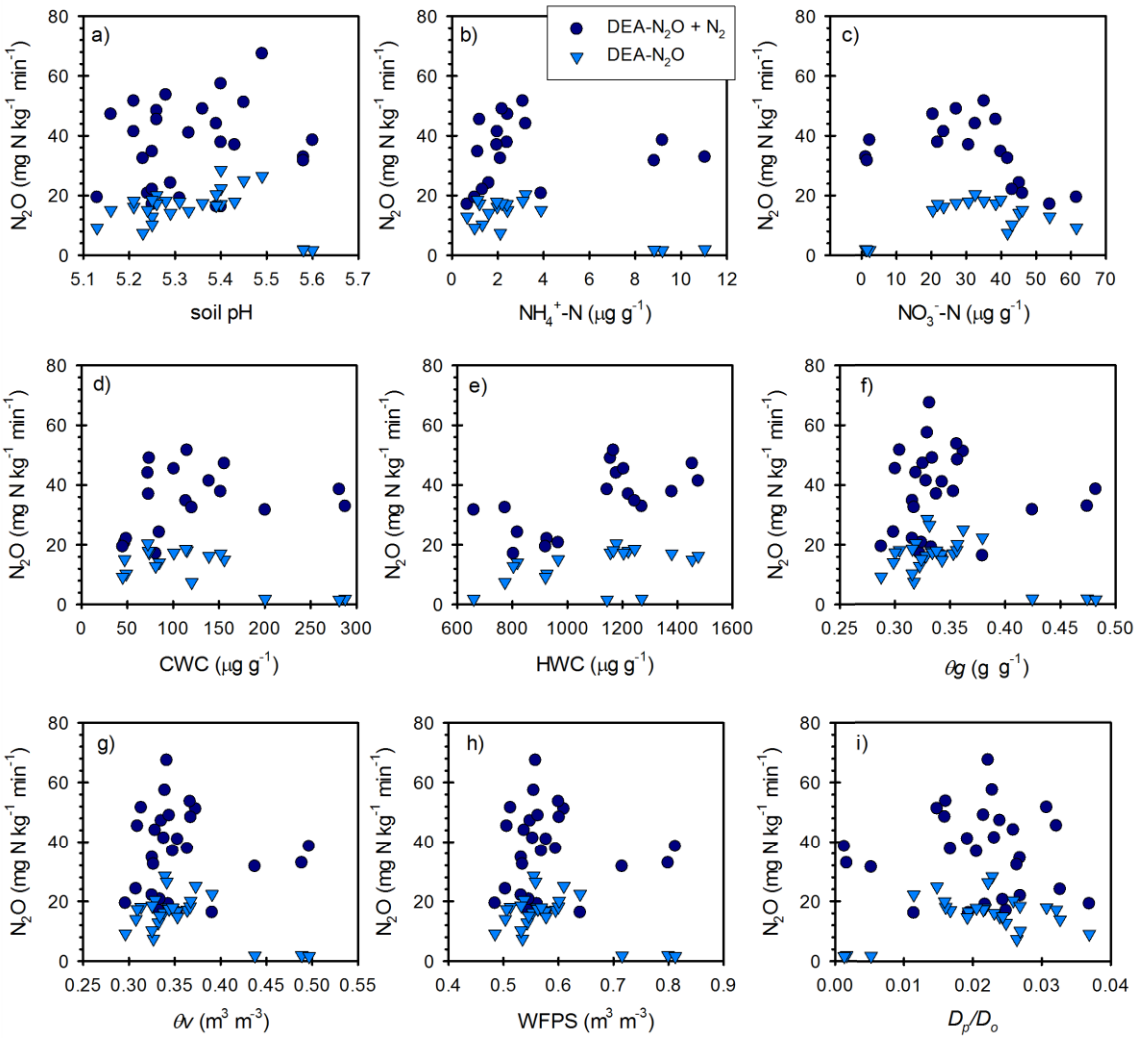
6.7 Supplementary Data

Supplementary Table S1 - Transformations for parametric statistics, where ʎ represents a box cox transform

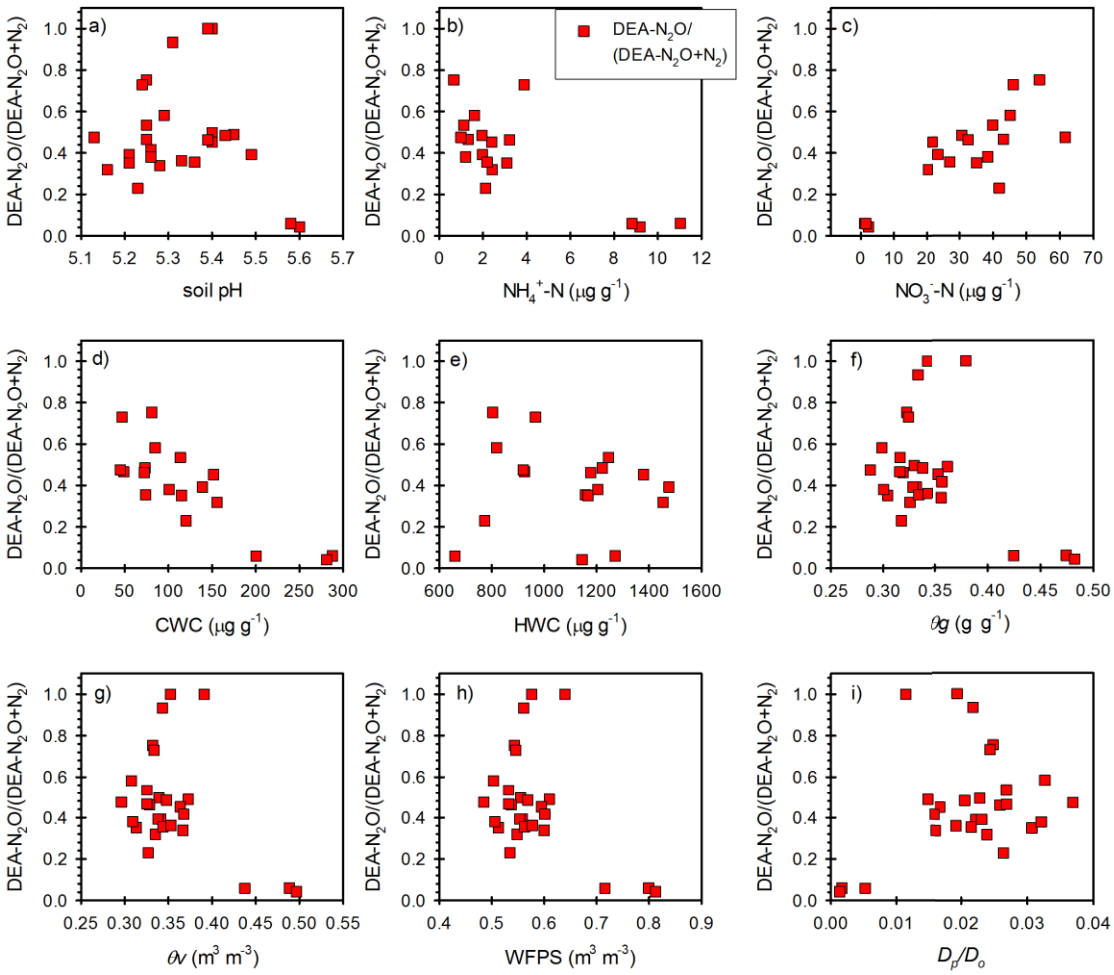
Variable	Transformation
Denitrification nitrous oxide + di-nitrogen (DEA-N_2O+N_2, mg N kg^{-1} min^{-1})	N/A
Denitrification nitrous oxide (DEA-N_2O, mg N kg^{-1} min^{-1})	N/A
Ratio of denitrification nitrous oxide and nitrous oxide + di-nitrogen (DEA-N_2O/(DEA-N_2O+N_2))	N/A
Soil pH	N/A
Gravimetric soil moisture (θg,)	ʎ = -2
Water-filled pore space (WFPS,)	N/A
Relative soil gas diffusivity (D_P/D_O)	N/A
Ammonium (NH_4^+-N, µg g^{-1})	ʎ = 1
Nitrate (NO_3^--N, µg g^{-1})	N/A
Cold water extractable carbon (CWC, µg g^{-1})	ʎ = 0
Hot water extractable carbon (HWC, µg g^{-1})	N/A



Supplementary Figure S1 - The nitrous oxide (N_2O) from the "with" and "without" acetylene (C_2H_2) incubations for each day of the experiment (DOE) since the wetting event.



Supplementary Figure S2 - Scatter plots showing the relationships between potential DEA-N_2O+N_2, and potential DEA-N_2O from a) soil pH, b) ammonium (NH_4^+-N), c) nitrate (NO_3^--N), d) cold water carbon (CWC), and e) hot water carbon (HWC), f) gravimetric soil moisture (θg), g) volumetric soil moisture content (θv), h) water-filled pore space (WFPS), and i) relative gas diffusivity (D_P/D_O)



Supplementary Figure S3 - Scatter plots showing the relationships between potential ratio $N_2O/(N_2O+N_2)$ from a) soil pH, b) ammonium (NH_4^+-N), c) nitrate (NO_3^--N), d) cold water carbon (CWC), and e) hot water carbon (HWC), f) gravimetric soil moisture (θg), g) volumetric soil moisture content (θv), h) water-filled pore space (WFPS), and i) relative gas diffusivity (D_P/D_O)

Chapter 7.

Diel Nitrous Oxide Fluxes and Nitrous Oxide Reductase in Soils With and Without Plants Under Constant Temperature

7.1 Introduction

Diel cycling of O_2 in pasture soil was observed *in situ* in Experiment 1 (Chapter 4) and Experiment 2 (Chapter 5). Diel cycling of soil O_2 is related to temperature driven changes in respiration (Lloyd and Taylor 1994). Diel changes to soil O_2 may influence nitrous oxide reductase (N_2OR) given that the O_2 concentration is a proximal controller of N_2OR (Knowles 1982). However, there may also be a diel cycling of N_2OR associated with changes to root exudates, which may also vary on a daily time step (Bahn et al. 2009). Diel cycling of N_2O fluxes have been identified (Christensen 1983, Smith et al. 1998, Das et al. 2012, Scheer et al. 2013). Deciphering the effects of root exudates, if any, on N_2OR may help interpret diel cycling of N_2O fluxes. The potential effects of root exudates on N_2OR may be assessed by comparing data collected from soils with plants and without plants over the course of a day from soils. Collecting this data in the absence of temperature change would cease temperature driven changes to soil O_2 resulting from respiration.

Plants deposit organic C and inorganic C into the rhizosphere soil via root decomposition and exudation (Marschner 1995). Diel cycling of delta (δ)$^{13}CO_2$ has been observed in grasslands which cannot be attributed to soil temperature or soil moisture, and are not related to CO_2 fluxes. This suggests the $\delta^{13}CO_2$ signal was from respiration of freshly assimilated C driven by photosynthesis (Bahn et al. 2009). Soil C from a plant's rhizosphere may influence the N_2O:N_2 ratio by altering the soil environment and affecting denitrification rates. However, daily change in soil O_2 from temperature driven respiration makes elucidation of plant root exudate effects on N_2OR difficult to establish.

Carbon is often limiting for denitrification in pasture soils (Saggar et al. 2012). There is a relationship between soil C sources, and the timing and magnitude of N_2OR (Morley et al. 2008), suggesting root exudates may influence N_2OR (Morley and Baggs 2010). No studies have linked diel C cycling associated with rhizosphere exudates in soils and N_2OR dynamics. Doing so may help explain the presence or absence of diel N_2O fluxes. One of the methods to assess the influence of

the rhizosphere is to compare planted soil with unplanted soil (Mezzari et al. 2011, Zhu et al. 2014).

The objectives of this experiment are to test i) whether N_2OR shows a diel cycle in response to changes in photosynthetically active radiation (PAR) while moisture and temperature are held constant, ii) if N_2O and N_2 fluxes and denitrification potential derived from denitrification enzyme assays (DEA) are greater from the soils with plants compared to soils without plants. It is hypothesized that i) N_2OR will vary on a daily time step, and ii) N_2O fluxes and potential N_2OR will be higher in soils with plants compared to soil without plants due to higher C concentrati ons from root exudates. It is expected that presence of root exudates will decrease soil O_2 due to greater respiration from plants, increase soil C concentrations due to rhizosphere deposition of root exudates, and increase the denitrification potential. In turn, these conditions will increase N_2O and N_2 fluxes because of greater substrates and denitrification, and decrease the N_2O:N_2 ratio by encouraging complete denitrification (Figure 7.1).

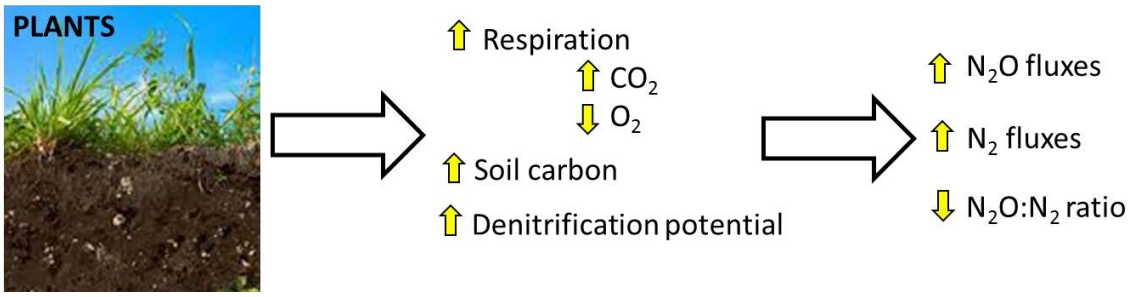


Figure 7.1 A graphical representation of the hypotheses that the presence of plants in soils will increase respiration rates, soil carbon and denitrification potential, and thus increase nitrous oxide and dinitrogen fluxes, and lower the ratio of those gases by promoting nitrous oxide reductase.

7.2 Methods

7.2.1 Plant and Soil Core Preparation

Soil was collected from the Lincoln Dairy Farm (-43° 38' 31.686" S, 172° 27' 54.0498" E). Soil was a stone-less silty loam with 15 - 35% clay. The soil is classified as a Mottled Immature Pallic Soil by the New Zealand Classification system (Hewitt 2010b). Soil was collected from the top 100 mm of the soil surface. Field moist soils were brought back to the lab and stored at 4°C overnight, then sieved to 2 mm. Soil cores were all repacked (section 3.2) to a bulk density of 1.0 Mg m^{-3} (height: 30 mm, i.d: 37.5 mm).

A factoral experimental design with two treatments was used; soils with plants ("plants") and soils without plants ("no plants").

Prior to soil collection, paspalum (*Paspalum dilatatum*) seed was sprouted on damp Whatman 42° filters placed in ziplock bags (Figure 7.5 a). Once sprouts were formed, five sprouts were planted per core into half the cores (Figure 7.5 b, c).

Both the "plant" and "no plant" cores were kept in a greenhouse for 10 weeks while the p aspalum established. Water was added to each of the cores at 5 or 10 mm at a time, twice a day.

Figure 7.2 A) Damp Whatman 42 filters where paspalum grass seeds were sprouted, and once sprouted, B) they were planted in repacked soil cores of the "plants" treatment, and C) left to grow.

7.2.1.1 Experimental Design

Once the plants were established, all soil cores were moved to growth cabinets on day of the experiment (DOE) -30, or 30 days before the start of gas sampling, to let the soils and plants equilibrate to the environmental conditions in the growth chamber. In the growth cabinets, cores were placed on a moisture retaining mesh and blocked, with 12 cores in each container (Figure 7.3 A).

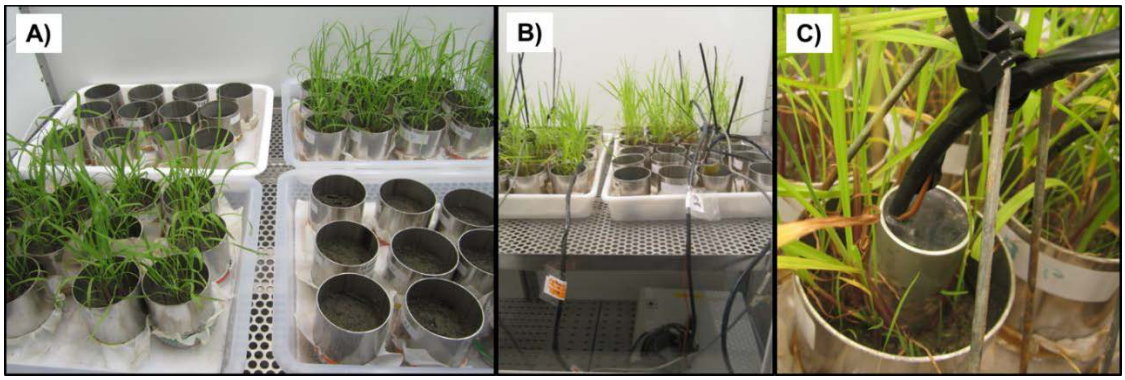


Figure 7.3 A) The cores were blocked in the growth cabinets, and B) two cores from each treatment were equipped with temperature and oxygen sensors C) which were placed in the center of the soil cores.

The soil cores were held at a constant soil moisture (80% WFPS) in both the "plant" and "no plant" treatments. Soil cores from each treatment were randomly allocated as being either a "gas" core used for gas flux measurements, or an "ancillary" core, used for destructive analyses at the end of the experiment.

7.2.1.2 Growth Cabinet Conditions

Continuous monitoring of soil oxygen concentration (O_2, SO-110, Apogee Instruments, Logan, UT, USA) and soil temperature (107 temperature probe, Campbell Scientific, Logan, UT, USA) were measured in two soil cores for each treatment. The ambient temperature and O_2 concentration within the growth chamber were also measured using one of each sensor mentioned above (Figure 7.3 B and C).

Once in the growth cabinets, the lights were turned on for 16 hours and turned off for 8 hours to mimic summer daylight conditions. Lights were turned on at hour 0 (to 700 µmol m^{-2} s^{-1} PAR), and turned off at hour 16.

Efforts were made to keep air temperature in the growth cabinet constant (25°C). To compensate for the heat generated from the lights, the temperature in the growth cabinet was turned down to 21°C when the lights were on, and the temperature was turned up to 25°C when the lights were off.

7.2.1.3 Nitrate Application

Four cores from each treatment were destructively sampled on DOE -3, and analysed for inorganic N concentrations(section 3.3). This was done to establish a N baseline that was considered when deciding how much NO_3^- to apply prior to gas sampling.

From here on, the day that the ^{15}N-labelled NO_3^- was added to the soil cores is called DOE 0. Thirty five µg of ^{15}N-labelled NO_3^--N was added as KNO_3^- g^{-1} soil to achieve 50 atom % enrichment. The same concentration of non-labelled NO_3^--N as KNO_3 was added to the ancillary cores at the same time. Nitrate was only added once at the beginning of the experiment.

7.2.1.4 N_2O and CO_2 sampling

On DOE 1, gas sampling started, which was 24 hours after the NO_3^--N was added to the soil. Every two hours, cores were incubated in 750 mL gas-tight jars equipped with a septa, in replicates of four for each treatment ("plants" and "no plants", one from each block). Gas samples were taken every 15 min for 45 min (Figure 7.4) for a total of four samples per incubation. Samples were collected with a glass syringe and analysed on the GC (section 3.4.2). The same gas sampling procedure was repeated again on DOE 3 for 24 hours.

7.2.1.5 Isotopic ^{15}N-N_2O and ^{15}N-N_2 gas samples

After sampling for N_2O and CO_2 concentrations, the cores were left sealed in the jars until they had been incubating for a total of four hours. Then, a 15 mL gas sample was extracted and immediately transferred to a 12 mL pre-evacuated (-1 atm) Exetainers® (Labco Ltd., United Kingdom) and run on the mass spectrometer to determine the ^{15}N enrichment of the N_2O and N_2 fluxes from the added NO_3^--N (section 3.4.1).

Because of the frequency of gas sampling, different sets of cores were used for successive incubations. There was a gap of 12 hours prior to a given soil core being reused for gas sampling (Figure 7.4).

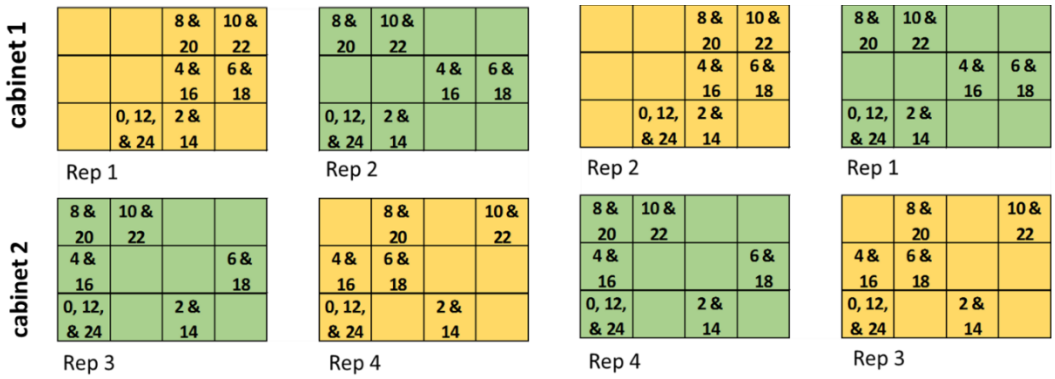


Figure 7.4 The experimental design showing the blocking for "plants" and "no plants" treatments, with each small square within the blocks representing one soil core. The numbers within the squares representing the cores are the hours during which the cores were incubated for gas sampling during the 24 hour sampling regime. The squares without numbers represent ancillary cores, or extra cores.

7.2.1.6 Ancillary Soil Sampling

Soil cores were destructively sampled for soil environmental analyses at the end of the experiment. After the last instance of gas sampling on DOE 3, four soil cores from each treatment were destructively sampled, and θg, inorganic N, organic C, soil pH, and electrical conductivity were measured (section 3.3). Volumetric water content and WFPS were calculated using θg and soil bulk density (section 3.1).

7.2.1.7 Relative Soil Gas Diffusivity

The relative soil gas diffusivity (D_P/D_O) was measured from four soil cores from each treatment as previously described (Rolston and Moldrup 2002, Balaine et al. 2013), and detailed in section 3.7.

7.2.1.8 Data and Analyses

Unless stated otherwise, data analyses were performed using Minitab (Minitab Inc. version 17 2010) with parametric statistics. Data were transformed, if needed, using box-cox transforms (Supplemental Table S1). If data were transformed, conclusions were drawn from the analysis on the transformed scale; however, figures present untransformed data. Differences between the no plant and plant treatments were tested with a two-tailed T-test using a critical value of $P < 0.05$. The difference between the means of the same variable on different days (DOE 1 and DOE 3) was tested using a pair T-test with a critical value of $P < 0.05$.

7.3 Results

7.3.1 Growth Chamber Environmental Conditions

Ambient O_2 concentrations averaged 20.7% in the growth chamber over the duration of the experiment (Figure 7.5 a).

The soil temperature from the "plant" and "no plant" soil cores increased by 2°C when the lights were on, compared to when the lights were off. However, the ambient temperature reflected the temperature adjustment made to compensate for the heat generated by the light, and thus showed the opposite trend compared to the soil temperature (Figure 7.5 b). Mean ambient temperature in the growth chamber was 23.7°C (± 0.04 SEM). The mean soil temperatures were 24.6°C (± 0.03 SEM) and 25.7°C (±0.07 SEM) in the "no plants" and "plants" treatments, respectively.

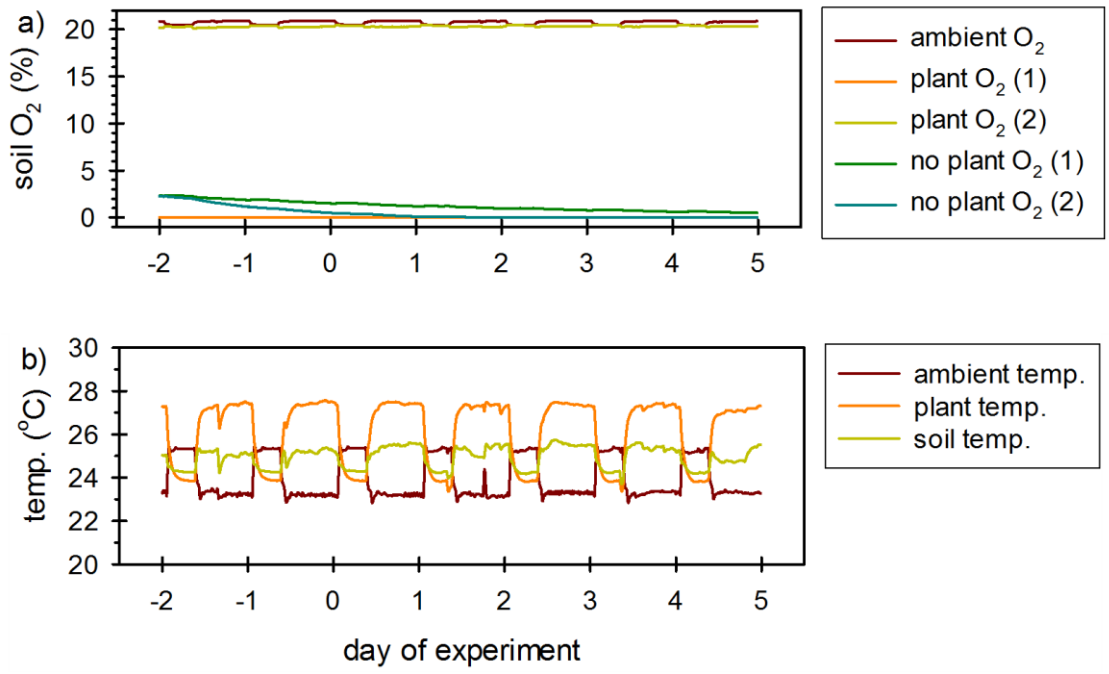


Figure 7.5 The soil O_2 and ambient O_2 concentrations (a) and soil temperature and air temperature (b)

7.3.2 Diel Cycling

No clear diel trend of CO_2 fluxes was observed from either treatment, on either DOE (Figure 7.6).

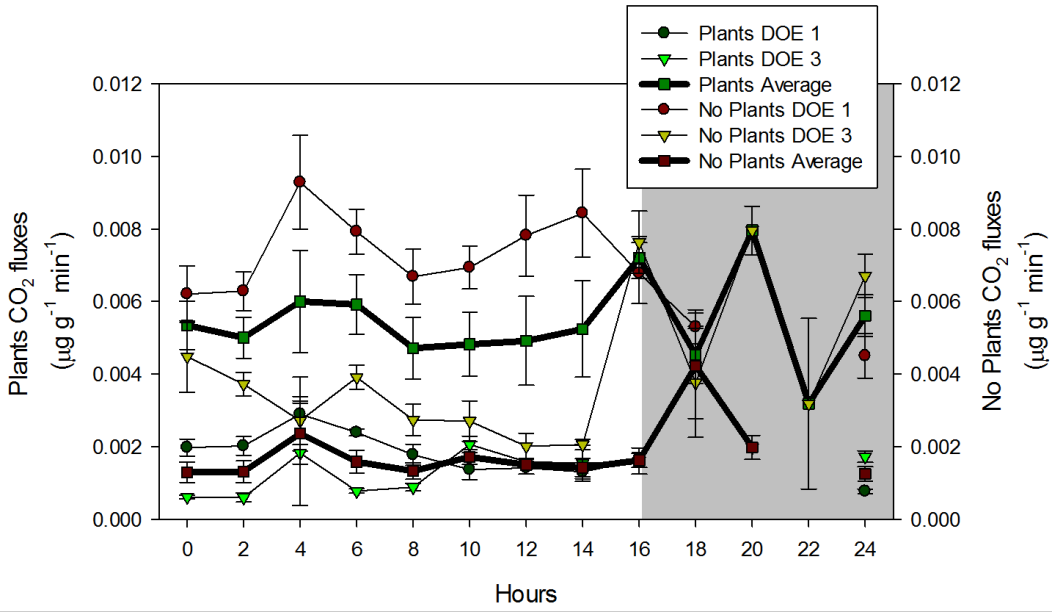


Figure 7.6 Carbon dioxide fluxes from day of the experiment (DOE) 1 and DOE 3 from "plants" and "no plants" treatment, and the average of both days over time (in bold). The shaded areas represent the time when the lights were off.

Nitrous oxide fluxes did not show any diel trends (Figure 7.7). There were spikes with high variability observed from the "no plant" treatment on DOE 1 at hour 12, and from the "plant" treatment on DOE 1 at hours 4 and 18 (Figure 7.7). Note the differences in scale between the "plant" and "no plant" N_2O fluxes in Figure 7.7.

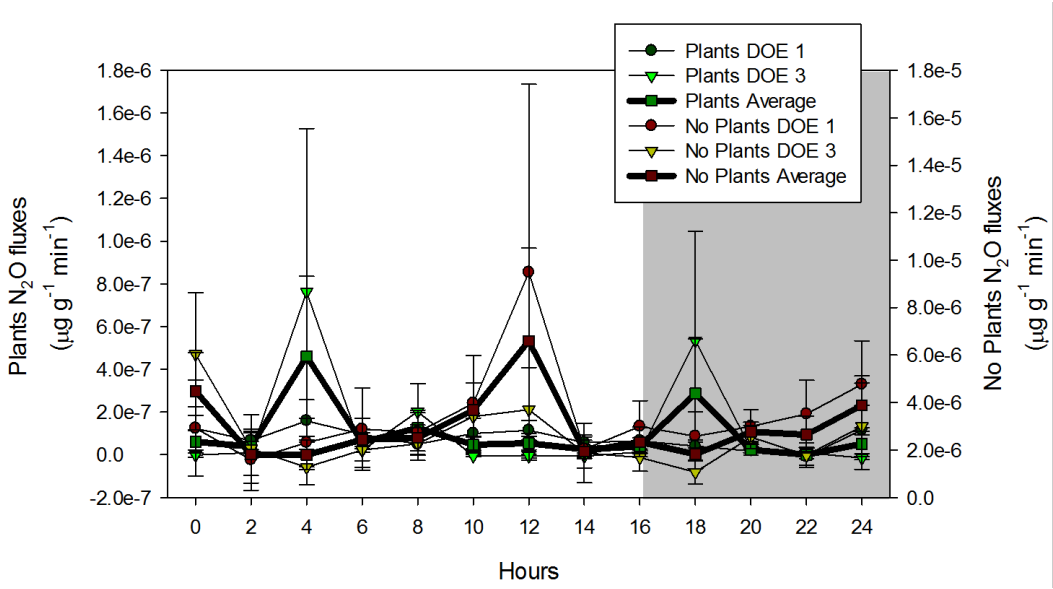


Figure 7.7 Nitrous oxide fluxes from day of the experiment (DOE) 1 and DOE 3 from "plants" and "no plants" treatment, and the average of both days (in bold). The shaded areas represent the time when the lights were off.

Nitrous oxide fluxes derived from the labelled NO_3^--N increased at hour 12 on both days in the "no plant" treatment (Figure 7.8 a, b), which was highly variable, and at hour 24 on DOE 3 (Figure 7.8 b). These changes were tracked by ^{15}N labelled N_2O (atom %) (Figure 7.8 b).

In the "plant" treatment, N_2O fluxes were high at the start of the experiment at hour 0 before decreasing on DOE 1, and there was a slight increase at hour 12 (Figure 7.8 c). There was an increase in N_2O concentration on hour 4 on DOE 3 from the "plant" treatment (Figure 7.8 d). As with the "no plant" treatment, in all instances, changes in N_2O fluxes in the plant treatment were tracked by changes in ^{15}N-N_2O (atom %).

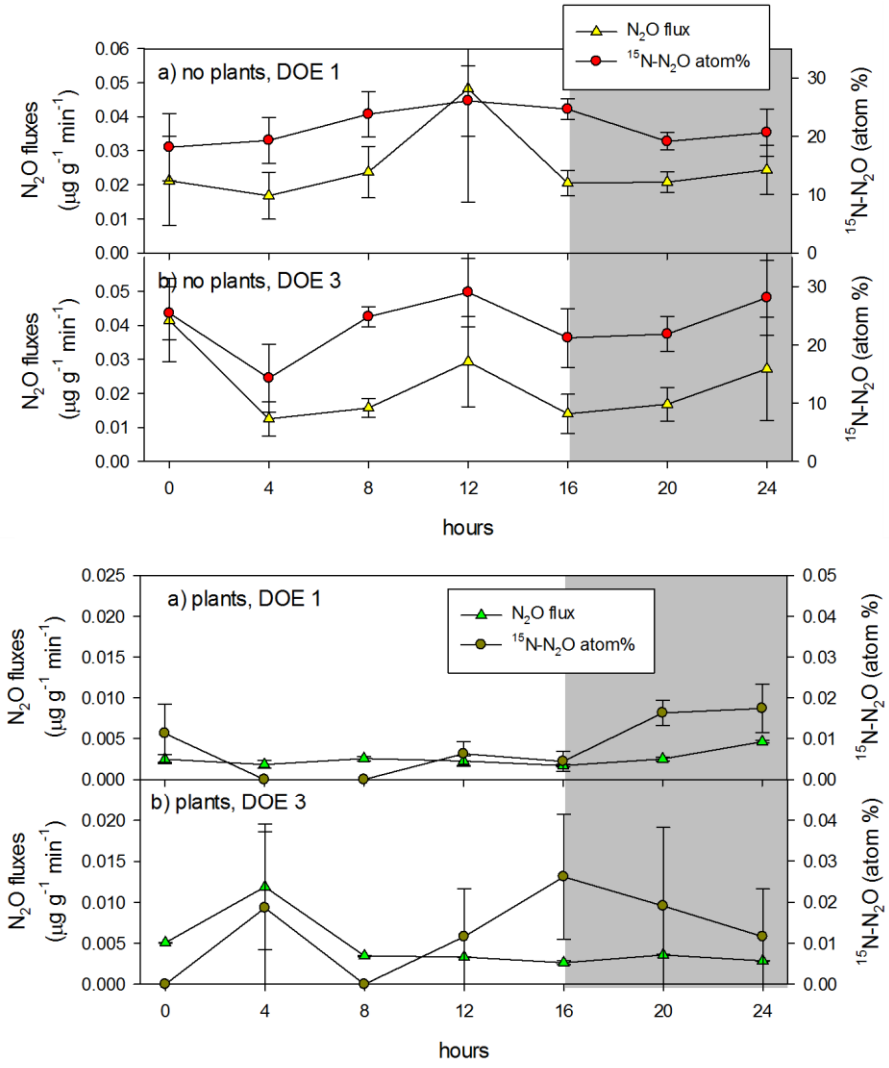


Figure 7.8 The nitrous oxide (N_2O) concentrations and ^{15}N labelled N_2O as atom % recovered from the "no plants" treatment on a) day of the experiment (DOE) 1 and b) DOE 3, and "plants" treatment on c) DOE 1 and d) DOE 3.

From the "no plants" treatment, the N_2 (atom %) was ≈ ambient during the experiment. The N_2 fluxes from this treatment increased at hour 16 and 20 on DOE 1 (Figure 7.9 a). The increase in N_2 fluxes at this time was not mimicked by the ^{15}N-N_2 (atom %). There was little variability on DOE 3 from both the N_2 fluxes at the ^{15}N-N_2 (atom %) (Figure 7.9 b).

From the "plants" treatment, there was an increase at hour 18 on both DOE 1 (Figure 7.9 c) and DOE 3 (Figure 7.9 d) in N_2 fluxes. In the "plants" treatment, the N_2 fluxes and the ^{15}N-N_2 (atom %) sometimes varied inversely to one another.

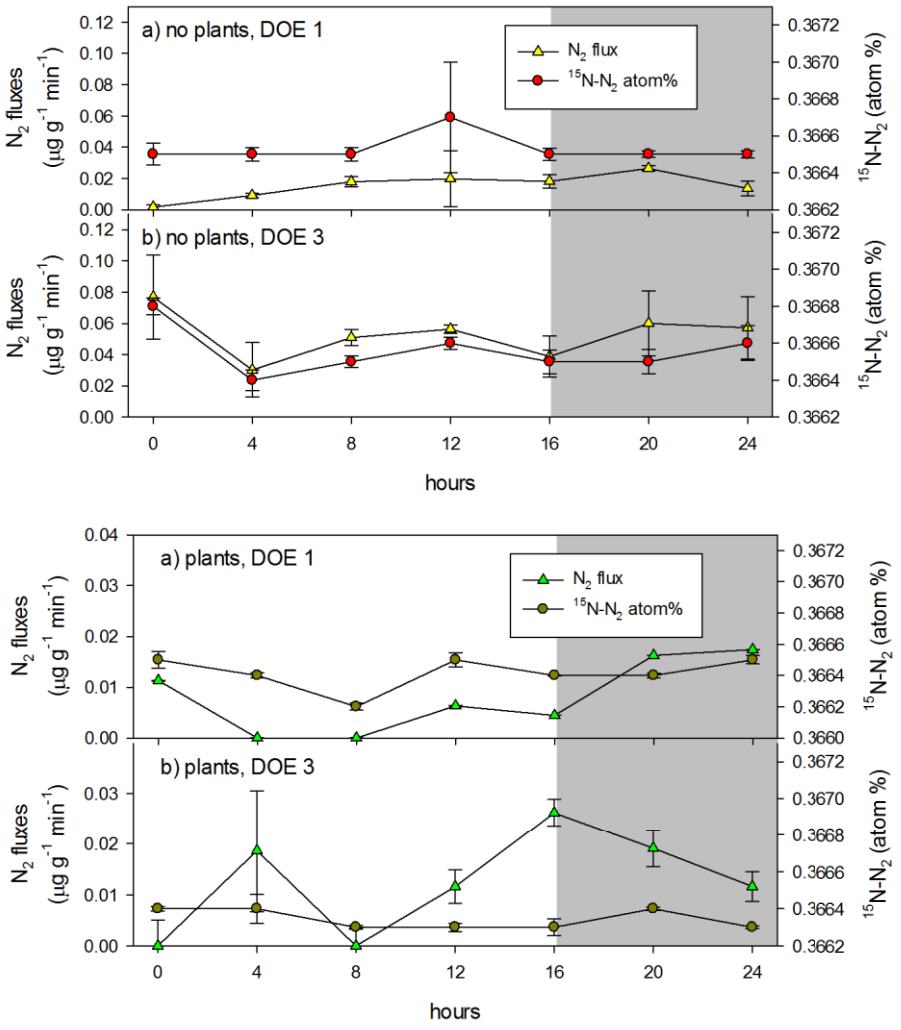


Figure 7.9 The dinitrogen (N_2) and ^{15}N-labelled N_2 as atom percent from the "no plants" treatment on a) day of the experiment (DOE) 1 and b) DOE 3, and "plants" treatment on c) DOE 1 and d) DOE 3.

In both the plants and no plants treatments, the ratio of N_2O/N_2 increased, peaking after 12 and 16 hours after the lights were turned on, respectively, before decreasing (Figure 7.10).

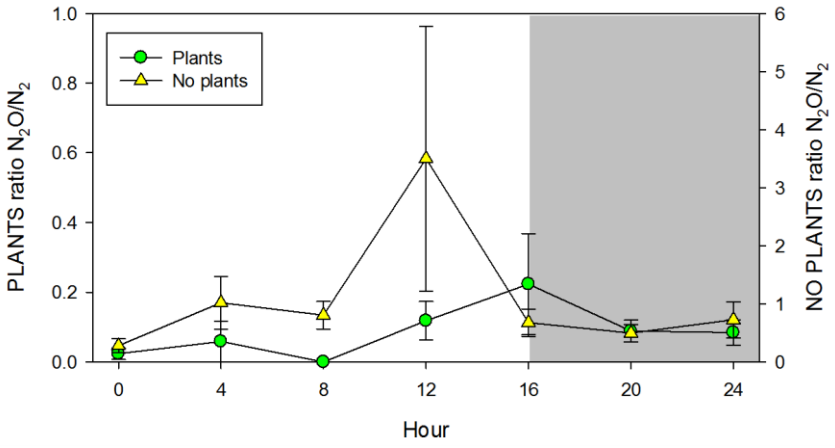


Figure 7.10 Mean and standard error of the ratio of N_2O/N_2 over 24 hours from the plants and no plants

7.3.3 Differences Between "Plants" and "No Plants" in Fluxes

Pooling the data from both sampling days, CO_2 fluxes, N_2O fluxes, ^{15}N-N_2O (atom %), N_2 fluxes, ^{15}N-N_2 (atom %) were 249, 2951, 92, 233, and 0.05% higher in the "no plants" treatment compared to the "plants" treatment, respectively (Table 7.1).

7.3.4 Soil Chemical and Nutrient Variables

There was no difference between the "plants" and "no plant" treatments with respect to CWC or HWC (Table 7.1). Soil pH did not differ between the "plants" and "no plants" treatments. Conductivity was 75% higher in the "no plants" treatment compared to the "plants" treatment (Table 7.1). Before the ^{15}N labelled NO_3^--N was added, NH_4^+-N was 230% higher in the "plants" treatment compared to the "no plants" treatment. Four days after the ^{15}N-labelled NO_3^--N was added, NH_4^+-N did not different between treatments (Table 7.1). While the NO_3^--N was generally higher in the "no plants" treatment, compared to the "plants" treatment, both before and after the ^{15}N-NO_3^--N addition, these differences were not statistically significant (Table 7.1).

7.3.5 Soil Hydrology, Oxygen, and Relative Soil Gas Diffusivity

There was no difference in θg, θv, and WFPS between the "plants" and "no plants" treatments (Table 7.1). Despite this lack of difference in soil hydrological variables, average soil O_2 was 94% higher in the "plants" compared to the "no plants" treatment (Table 7.1). The soil O_2 concentration within the individual "no plants" cores were 1.9 and 1.6%. The soil O_2 in the "plants" cores varied between 0% and 20.3%. Contrasting the O_2 measurements, D_P/D_O was 101% lower from the "plants" treatment compared to the "no plants" treatment (Table 7.1).

7.3.6 Denitrification Potential

Overall denitrification potential, as DEA-N_2O and DEA-N_2O+N_2, which were run at the end of the experiment, were 50 and 668% higher from the "plants" treatment compared to the "no plants" treatment, respectively (Table 7.1). Over the course of the 48 hour DEA incubation, DEA-N_2O+N_2 increased more rapidly in the "plants" treatment compared to the "no plants" treatment (Figure 7.11 a, b). These differences in DEA-N_2O+N_2 and DEA-N_2O between treatments are reflected in the ratio of DEA-N_2O/(DEA-N_2O+N_2) (Figure 7.11 c). The overall mean ratio of DEA-N_2O/(DEA-N_2O+N_2) was 80% lower in the "plants" treatment compared to the "no plants" treatment (Table 7.1).

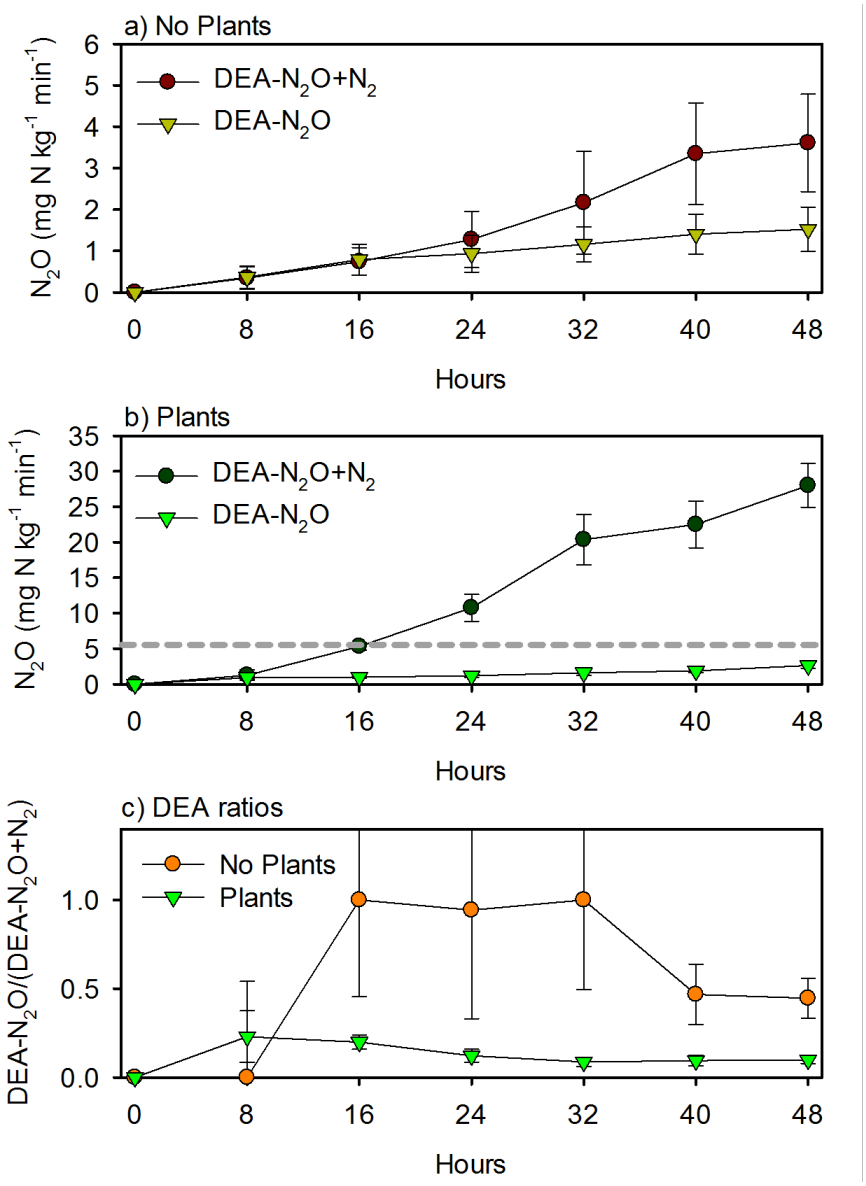


Figure 7.11 Changes in DEA-N_2O+N_2 and DEA-N_2O over time during the 48 hour incubation time for denitrification enzyme assays. Note the differences in scale between the "no plants" and "plants". The dashed line on graph "b" represents the extent of the scale of the graph of "a". c) The mean ratio of DEA-N_2O/(DEA-N_2O+N_2) from the denitrification enzyme assays from the "plants" and "no plants". The error bars represent the standard error of the means.

Table 7.1 Mean and standard error of the means (SEM) using data from both days of data collection, and the T-test statistic P-values comparing the means from both treatments.

Variable	No Plants		Plants		
	Mean	± SEM	Mean	± SEM	P-value
GC - nitrous oxide fluxes (mg N kg^{-1} min^{-1})	2.99 $x10^{-6}$	3.9 $x10^{-7}$	9.8 $x10^{-8}$	3.7 $x10^{-8}$	0.000
GC - carbon dioxide fluxes (mg kg^{-1} min^{-1})	1.55 $x10^{-3}$	1.6 $x10^{-4}$	5.41 $x10^{-3}$	2.9 $x10^{-4}$	0.000
nitrous oxide fluxes (mg N kg^{-1} min^{-1})	0.02375	2.74 $x10^{-3}$	3.631 $x10^{-3}$	6.88 $x10^{-4}$	0.000
^{15}N-nitrous oxide fluxes (atom %)	22.58	1.10	1.901	0.633	0.000
dinitrogen fluxes (mg kg^{-1} min^{-1})	0.03405	6.08 $x10^{-3}$	0.01021	2.30 $x10^{-3}$	0.002
^{15}N- dinitrogen fluxes (atom %)	0.36655	2.8 $x10^{-5}$	0.36638	1.9 $x10^{-5}$	0.000
before exp. nitrate-N (µg g^{-1})	5.38	2.35	0.74	0.16	0.143
before exp. ammonium-N (µg g^{-1})	1.29	0.19	4.28	0.73	0.028
after exp. nitrate-N (µg g^{-1})	20.67	7.86	16.05	8.52	0.707
after exp. ammonium-N (µg g^{-1})	3.99	1.37	4.54	2.03	0.833
cold water carbon (µg g^{-1})	237.2	18.6	275.2	48.8	0.520
hot water carbon (µg g^{-1})	577.7	34.2	546.0	31.1	0.523
soil pH	5.97	0.05	5.95	0.04	0.792
conductivity (% soluble salts)	18.46	1.86	10.52	0.83	0.017
gravimetric soil moisture	0.46	0.01	0.47	0.01	0.575
volumetric water content	0.48	0.01	0.49	0.01	0.575
water-filled pore space	0.79	0.01	0.80	0.02	0.575
relative soil gas diffusivity	0.22	0.07	0.11	0.02	0.002
Soil oxygen	0.76	0.02	10.17	0.34	0.000
DEA-N_2O (mg N kg^{-1} min^{-1})	5.41	1.52	4.28	1.82	0.054
DEA-N_2O+N_2 (mg N kg^{-1} min^{-1})	13.01	4.75	58.47	13.93	0.639
DEA-N_2O/(DEA-N_2O+N_2)	0.47	0.11	0.07	0.02	0.034

7.4 Discussion

Contrary to the original hypothesis, there was no apparent diel cycling of N_2OR in the "plant" treatment driven by PAR. It is difficult to tell, based on the results, whether this is because of suboptimal soil O_2 conditions for N_2OR production in the "plants" treatment, or because of a lack of N_2OR response in the absence of temperature change.

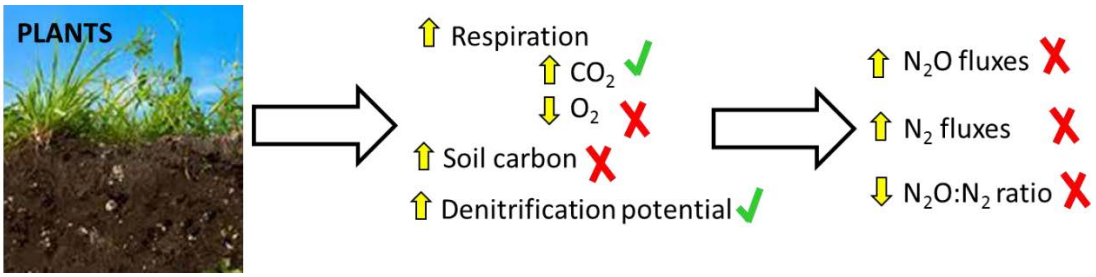


Figure 7.12 A graphical representation of the results according to the original hypothesis that the presence of plants in soils will alter soil and biological characteristics therefore influencing nitrous oxide fluxes, dinitrogen fluxes, and the ratio of those gases.

The diel ratio of N_2O:N_2 fluxes suggest changes in N_2OR in the soil during this experiment were not increased by the presence of plants; there was a lower N_2O:N_2 ratio and less variability in the N_2O:N_2 ratio in the "plants" treatment compared to the "no plants" treatment. The observed increases in N_2OR activity after 12 and 16 hour of the lights being on in "no plants" and "plants" treatments, respectively (Figure 7.10), cannot be directly attributed to increases in soil temperature, which can affect N_2O and N_2 fluxes (Phillips et al. 2015), since the soil temperature did not change significantly while the lights were on (Figure 7.5). If this N_2O:N_2 pattern is not random, the cause, if any, to increases and decreases over the course of the 24 sampling regimes were also not related to respiration, as there was no diel patterns to CO_2 fluxes. Future studies should explore how other factors that can influence N_2OR, such as micro-pore O_2 dynamics and nitrate, vary on a diel scale.

Contrary to the original hypothesis, soil C was not higher in the soil with plants compared to the soil without plants. Soil C increase in the presence of the rhizosphere can be due to the decomposition of dead roots (Marschner 1995). Given the short-lived nature of the experiment (16 weeks), it is unlikely that there was mass turnover of roots in the soil, thus there would be little contribution to the soil C pool from root decomposition. Assuming the lack of difference in soil C between treatments was not an artefact of the experimental design (i.e. sieving and repacking soil), then the results indicate plant root exudation did not make a significant contribution to HWC or CWC concentrations by the end of the experiment. However, it could also mean that root exudate C was transient and was utilized prior to soil extraction. Carbon based exudates expelled from roots may have been used during respiration, and thus been unavailable for denitrification. Carbon respiration has been noted independent of temperature or moisture change (Bahn et al. 2009).

Different plant species have different root exudate compositions, thus the form of C from the paspalum root exudates, or the behaviour of the paspalum rhizosphere root exudation on a daily time scale in relation to changes in PAR, may not have been favourable to the experimental conditions. Measuring changes in root exudate composition and concentrations over the course the day was outside of the scope of this experiment, but could be explored in future studies. It has been found that different forms of C in combination with difference O_2 concentrations affect NO_3^- reduction differently (Morley and Baggs 2010) suggesting a different result may be achieved under the same experimental conditions but with a different plant species.

If the root exudates are not transient, the higher respiration rate in the "plants" treatment was most likely due to respiration from plants and not the soil since there was no difference soil C content between the treatments. Despite higher rates of respiration, soil O_2 concentrations as measured by the Apogee sensors were higher in the "plants" compared to the "no plants", contrasting the respiration results. The higher O_2 concentrations in the "plants" compared to the "no plants" treatment, despite equivalent soil moistures, is indicative of the changes in soil structure due to growing plants in the repacked soil cores (Figure 7.13). The plants made the soil cores more porous. The implications of this are that at equivalent soil moistures, the planted soil cores would be more diffusive due to the greater pore size and connectivity than the soil in the unplanted cores.

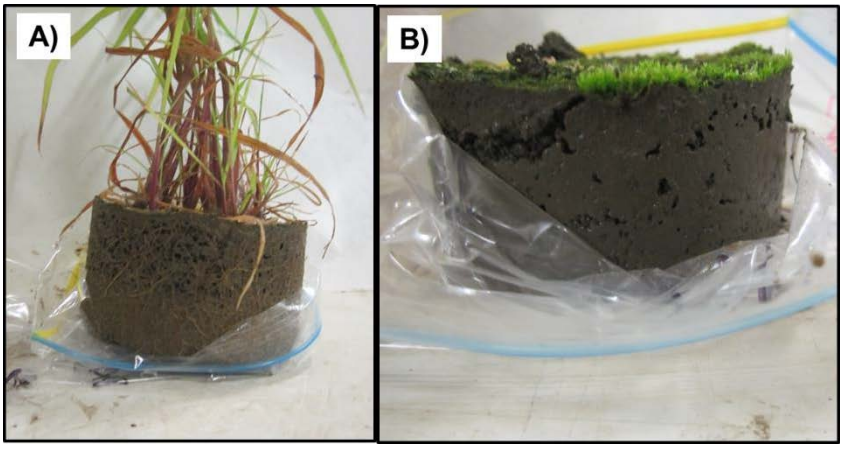


Figure 7.13 The differences in soil structure in the soils with plants (A) and the soils without plants (B)

The D_P/D_O measurements showed lower diffusivity in the "plants" treatment compared to the "no plants" treatment despite the differences in soil structure leading to greater pore sizes in the "plants" treatment, noted above. The lower D_P/D_O measurements in the plant cores may have

been representative of the short time over which the D_P/D_O measurements are made (2 hours). They suggest that O_2 uptake from C respiration in the presence of plants slowed - but did not outpace - O_2 diffusion into the planted soil cores. The D_P/D_O measurements may not accurately represent O_2 diffusion dynamics in the planted cores because the D_P/D_O measurements are supposed to be only physical representations of O_2 diffusion rates and assume biological activity is negligible (Rolston and Moldrup 2002). However, the high CO_2 production from the "plant" cores infers biological activity (photosynthesis) resulted in O_2 uptake. Future research is required to couple respiration and D_P/D_O to better understand how they work together to represent soil O_2 availability and movement through soil (Petersen et al. 2013).

For N_2O to be reduced to N_2, D_P/D_O must approach a value of 0.006 (Balaine et al. 2013, Balaine et al. 2016). Differences in experimental conditions, such as greater soil moisture, may have helped achieve lower D_P/D_O. Future studies should consider doing a moisture retention curves to ensure the matric potential is greater than the air-entry value, which has been found to be significant for N_2O and N_2OR production (Balaine et al. 2013). This would ensure O_2 conditions for N_2OR are appropriate in the soil. Thus, the implications of the relatively high D_P/D_O are that soil conditions were not optimal for significant N_2OR production in either treatment (Stepniewski 1981).

While there were no differences in soil C between the "plants" and "no plants" treatments, and N_2O and N_2 fluxes did not increase in the presence of plants, denitrification potential was greater in the planted soil compared to the soil without plants. This result suggests the presence of the rhizosphere cultivated microbes with denitrification capabilities, consistent with previous research (Rouatt et al. 1960, Hall et al. 1998, Rovira 1965, Foster 1988, Smalla et al. 2001). There was likewise lower DEA-N_2O/(DEA-N_2O+N_2), or higher potential N_2OR, in the "plant" treatment compared to the "no plant" over the course of the 3 day experiment. However, N_2OR is highly sensitive to the presence of O_2 (Wrage et al., 2001), and it has previously been established that soil O_2 during this experiment was not low enough for high N_2 production in either treatment. This is supported by the low ^{15}N recovery as ^{15}N-N_2 which suggests little to no N_2OR activity from the ^{15}N-labelled NO_3^--N addition from either treatment. The relatively higher N_2O fluxes coupled with the higher recovery of ^{15}N as ^{15}N-N_2O in the "no plants" treatment shows that the added ^{15}N-labelled NO_3^--N was the dominant substrate used to produce N_2O fluxes during the gas sampling. Of the 35 μg g^{-1} of ^{15}N-labelled NO_3^--N added, ~20 μg g^{-1} was lost in both "plants" and "no plants" between the before and after the addition. In the "no plant" treatment, the higher recovery of ^{15}N-N_2O means that these N_2O emissions can be attributed to either denitrification or nitrifier-

denitrification (Wrage et al. 2001, Zhu et al. 2013). The low recovery of $^{15}N\text{-}N_2O$ and $^{15}N\text{-}N_2$ from the "plant" treatment suggests that given the sub-optimal conditions for N_2O production, the added NO_3^--N could have been either leached out the bottom of the cores, or was taken up by plants.

7.5 Conclusion

The differences between the "plants" and "no plants" treatments confer the complicated story; while the presence of plants leads to greater potential for denitrification from the soils with plants, higher N_2O fluxes were observed from the "no plants" treatment. In summary, the lack of any consistent diel N_2OR from both the "plants" and "no plants" treatments can be attributed to unfavourable soil conditions. This may be partially due to the lack of diel changes in soil O_2 in the absence of diel temperature changes. This, along with the confounding effects of soil moisture status, denitrification potential, soil nutrients, and soil O_2 dynamics, made creating optimal conditions for N_2OR in both treatments difficult.

The results from this study reinforces the importance of considering how plants affect the soil environment, and has implications for transferring results obtained in the laboratory soil experiments without plants, to the field.

7.6 References

Bahn, M., M. Schmitt, R. Siegwolf, A. Richter, and N. Brüggemann. 2009. Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale? The New Phytologist 182:451-460.

Balaine, N., T. Clough, M. Beare, S. Thomas, and E. Meenken. 2016. Relative gas diffusivity controls on soil nitrous oxide and dinitrogen fluxes and their ratios. Soil Science Society of America. DOI: 10.2136/sssaj2015.09.0350

Balaine, N., T. Clough, M. Beare, S. Thomas, E. Meenken, and J. Ross. 2013. Changes in Relative Gas Diffusivity Explain Soil Nitrous Oxide Flux Dynamics. Soil Science Society of America Journal 77:1496-1505.

Christensen, S. 1983. Nitrous oxide emission from a soil under permanent grass: Seasonal and diurnal fluctuations as influenced by manuring and fertilization. Soil Biology and Biochemistry 15:531-536.

Das, B. T., K. Hamonts, E. Moltchanova, T. J. Clough, L. M. Condron, S. A. Wakelin, and M. O'Callaghan. 2012. Influence of photosynthetically active radiation on diurnal N_2O emissions under ruminant urine patches. New Zealand Journal of Agricultural Research 55:319-331.

Foster, R. C. 1988. Microenvironments of soil microorganisms. Biology and Fertility of Soils 6:189-203.

Hall, J. M., E. Paterson, and K. Killham. 1998. The effect of elevated CO_2 concentration and soil pH on the relationship between plant growth and rhizosphere denitrification potential. Global Change Biology 4:209-216.

Hewitt, A. E. 2010. New Zealand Soil Classification. Manaaki-Whenua Press.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Lloyd, J., and J. A. Taylor. 1994. On the Temperature Dependence of Soil Respiration. Functional Ecology 8:315-323.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. 2nd edition. Elsevier.

Mezzari, M., Z. Daniela Mara Hoffmann, H. Corseuil, and A. Nogueira. 2011. Potential of grasses and rhizosphere bacteria for bioremediation of diesel-contaminated soils. Revista Brasileira de Ciência do Solo 35:2227-2236.

Minitab Inc. version 17. 2010. Minitab Statistical Software [Computer software]. State College, PA.

Morley, N., E. Baggs, P. Dörsch, and L. Bakken. 2008. Production of NO, N_2O and N_2 by extracted soil bacteria, regulation by NO_2^- and O_2 concentrations. FEMS Microbiology Ecology 65:102-112.

Morley, N., and E. M. Baggs. 2010. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biology and Biochemistry 42:1864-1871.

Petersen, S., P. Ambus, L. Elsgaard, P. Schjønning, and J. Olesen. 2013. Long-term effects of cropping system on N_2O emission potential. Soil Biology and Biochemistry 57:706-712.

Phillips, R. L., A. M. S. McMillan, T. Palmada, J. Dando, and D. Giltrap. 2015. Temperature effects on N_2O and N_2 denitrification end-products for a New Zealand pasture soil. New Zealand Journal of Agricultural Research 58:89-95.

Rolston, D. E., and P. Moldrup. 2002. Gas diffusivity. Pages 1113-1139 in J. H. Dane and G. C. Topp, editors. Methods of Soil Analysis, part 4, Physical Methods. Soil Science Society of America, Madison, Wisconsin.

Rouatt, J. W., H. Katznelson, and T. M. B. Payne. 1960. Statistical evaluation of the rhizosphere effect. Soil Science Society of America Proceedings 24:271-273.

Rovira, A. D. 1965. Interactions Between Plant Roots and Soil Microorganisms. Annual Review of Microbiology 19:241-266.

Saggar, S., N. Jha, J. Deslippe, N. S. Bolan, J. Luo, D. L. Giltrap, D. G. Kim, M. Zaman, and R. W. Tillman. 2012. Denitrification and N_2O:N_2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. Science of the Total Environment 465:22.

Scheer, C., P. R. Grace, D. W. Rowlings, and J. Payero. 2013. Soil N_2O and CO_2 emissions from cotton in Australia under varying irrigation management. Nutrient Cycling in Agroecosystems 95:43-56.

Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, and G. Berg. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology 67:4742-4751.

Smith, K. A., P. E. Thomson, H. Clayton, I. P. McTaggart, and F. Conen. 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmospheric Environment 32:3301-3309.

Stepniewski, W. 1981. Oxygen diffusion and the strength as related to soil compaction. II Oxygen diffusion coefficient. Polish Journal of Soil Science 14: 3-13.

Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry 33:1723-1732.

Zhu, B., J. Gutknecht, D. Herman, D. Keck, M. Firestone, and W. Cheng. 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology and Biochemistry 76:183-192.

Zhu, X., M. Burger, T. Doane, and W. Horwath. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proceedings of the National Academy of Sciences 110:6328-6333.

Chapter 8. Differences in Nitrous Oxide Reductase Enzymes and Denitrification Potential from Rhizosphere and Bulk Soil in a Grazed and Irrigated Paddock

8.1 Introduction

Previous *in situ* studies in this thesis took place on grass pastures. Laboratory studies often use soils without plants because more control over the experimental conditions can be obtained. However, in Experiment 3, Chapter 6, the denitrification potential was higher from soils with plants compared to soils without plants alluding to the biological differences in soil caused by the presence of the rhizosphere. This chapter seeks to explore how soil affected by plants (rhizosphere soil) differs in N_2OR activity compared to soil not affected by plants (bulk soil).

The soil environment differs biologically and chemically between the rhizosphere and bulk soil (Table 8.1). These differences are expected to be more pronounced closer to the root, with soil surrounding a root exhibiting longitudinal and radial gradients away from the root system (Sørensen 1997). Rhizosphere soil has greater microbial loads (Foster 1988) and microbial diversity (Rovira 1965, Smalla et al. 2001), less aerobic bacteria (Garbeva et al. 2004), higher respiration rates (Garbeva et al. 2004) and higher denitrification rates (Rouatt et al. 1960, Hall et al. 1998). These biological differences between the bulk and rhizosphere soil relate to the chemical effects that the rhizosphere has on the adjacent soil environment. Plant respiration may reduce O_2 levels in the rhizosphere soil (Garbeva et al. 2004), while hydrogen (H^+) or bicarbonate (HCO_3^-) from the rhizosphere can increase soil pH (Nye 1981). The expulsion of root exudates can alter soil organic carbon (C) and inorganic nitrogen (N) concentrations (Lynch and Whipps 1990, Cheng et al. 2003, Philippot et al. 2009, Zhu et al. 2014). These chemical factors can influence biological denitrification rates and the gaseous end-products emitted during denitrification (Table 8.2). Dinitrogen (N_2) fluxes from soil are difficult to measure directly because of the high ambient concentrations. However, it is important to understand N_2 dynamics, along with N_2O, because together, along with NH_3 and NO_x, they help inform total gaseous N losses from the soil system.

Table 8.1 Chemical and biological differences between rhizosphere and bulk soils

Variable	Rhizosphere	Bulk	Other notes
Organic Carbon		>	
Inorganic Nitrogen		>	
NO_3^-		>	increases pH
NH_4^+		>	lowers pH
Microbial Diversity		>	
Anaerobic Bacteria		>	
Respiration Rates		>	
Denitrification		>	

Table 8.2 The effects of changing soil chemical factors on denitrification rates, and nitrous oxide reductase (N_2OR), which will affect the proportion of N_2O or N_2 produced during denitrification. The arrows represent the direction of the change

	Variable	Denitrification rates	N_2O	N_2OR	N_2
↑	Inorganic nitrogen	↑	↑	↓	↓
↑	Organic carbon	↑	↓	↑	↑
↑	pH	↑	↑	↑	↑

The rhizosphere has no well-defined boundary (Hinsinger et al. 2005). Effective separation of bulk and rhizosphere soil can be achieved when a single plant is grown (Prendergast-Miller et al. 2014). Often, plants are shaken to separate bulk and rhizosphere with loose soil considered to be bulk soil, and soil attached to the roots considered to be rhizosphere (Højberg et al. 1996, Prendergast-Miller et al. 2014). Some studies have used planted and unplanted soils to assess how the rhizosphere affects microbial populations and communities (Mezzari et al. 2011, Zhu et al. 2014). Despite the various methods used, there is no standardized protocol for bulk and rhizosphere separating soil.

Field studies are complicated by spatial variability. This can be especially challenging in grazed pastures. Grazing can induce compaction (Ruser et al. 1998, Ruser et al. 2006, Bhandral et al. 2007), and excretal returns to soil from grazing animals can result in localized areas of increased soil N concentration which contribute to spatial variability of nutrients (Moir et al. 2011). Spatial variability inherent in managed systems is often overlooked by compositing samples. However, quantifying spatial variability may have important implications for development of management strategies from N_2O emissions in managed systems, and could inform future sampling campaigns.

The objectives of this experiment were to: 1) determine if N_2O and N_2 fluxes are higher, and N_2O:N_2 ratios are lower, from the rhizosphere soil compared to the bulk soil, and 2) determine if there are spatial differences in N_2O and N_2 fluxes between different paddocks. It was hypothesized that the rhizosphere soil would have higher N_2O and N_2 production compared to the bulk soil due to higher soil nutrient concentrations in the rhizosphere soil. The ratio of N_2O:N_2 is expected to be lower in the rhizosphere soil compared to the bulk soil, due to higher C in the rhizosphere soil, which will favour N_2OR.

8.2 Methods

8.2.1 Study Site

Soil was collected from Beacon farm, the site of Experiment 1, a commercial dairy farm located on the Canterbury Plains. The soil and pasture information is detailed in section 4.3.1.

The pasture at this site is irrigated with a central pivot irrigator. The paddocks are fenced in to "pie slices" and each slice is grazed by dairy cattle herd on an approximately 21 day rotation. Two sites were randomly selected from within three paddocks for a total of six sites (Figure 8.1). From each of these sites, bulk and rhizosphere soils were collected on March 9, 2015.

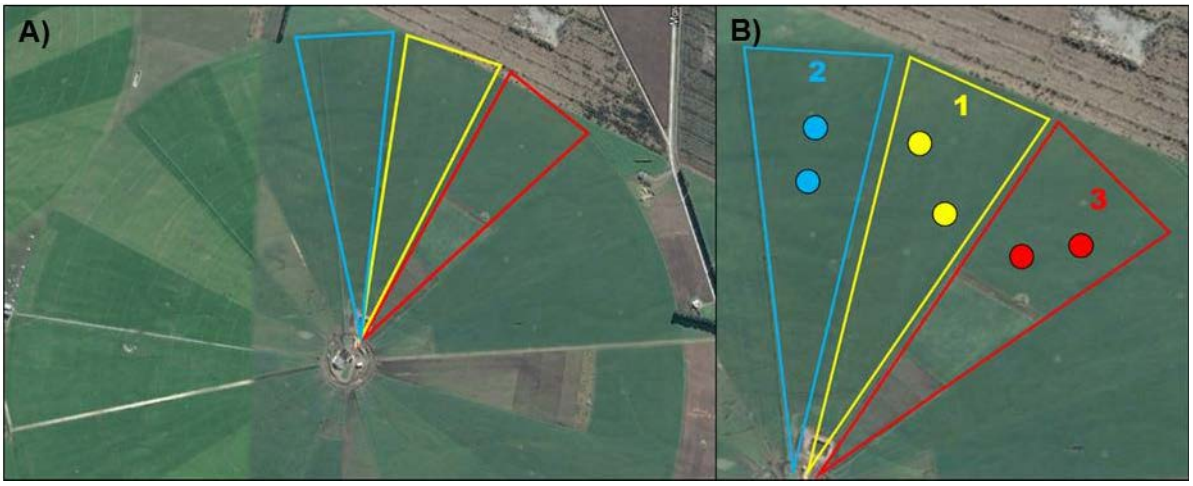


Figure 8.1 Spatial representation of Beacon Farm (A), and the locations of soil collection within each paddock (B)

8.2.2 Soil Collection

To collect soil for this study, a ≈ 300 by 300 mm area, and 100 mm depth of pasture turf and soil was excavated from each sampling location (Figure 8.2). The pasture turf was placed in a bucket and gently shaken. Any soil that fell freely off was considered to be bulk soil. The bulk soil, and the remaining slab of pasture, were separately bagged and immediately brought back to the lab in a chilly bin. In the lab, soil from the rhizosphere was pulled directly off the roots. Both the bulk soil and the rhizosphere soil were then sieved to 2 mm, and any visible plant material was removed from the soils (Figure 8.2). A subsample of the soils was shipped cool via overnight courier to Landcare, Palmerston North, and stored at 4°C until analysis. This soil was incubated to determine N_2O and N_2 fluxes. The rest of the soil was kept at Lincoln University and used for denitrification enzyme assay and to determine ancillary soil chemical data.

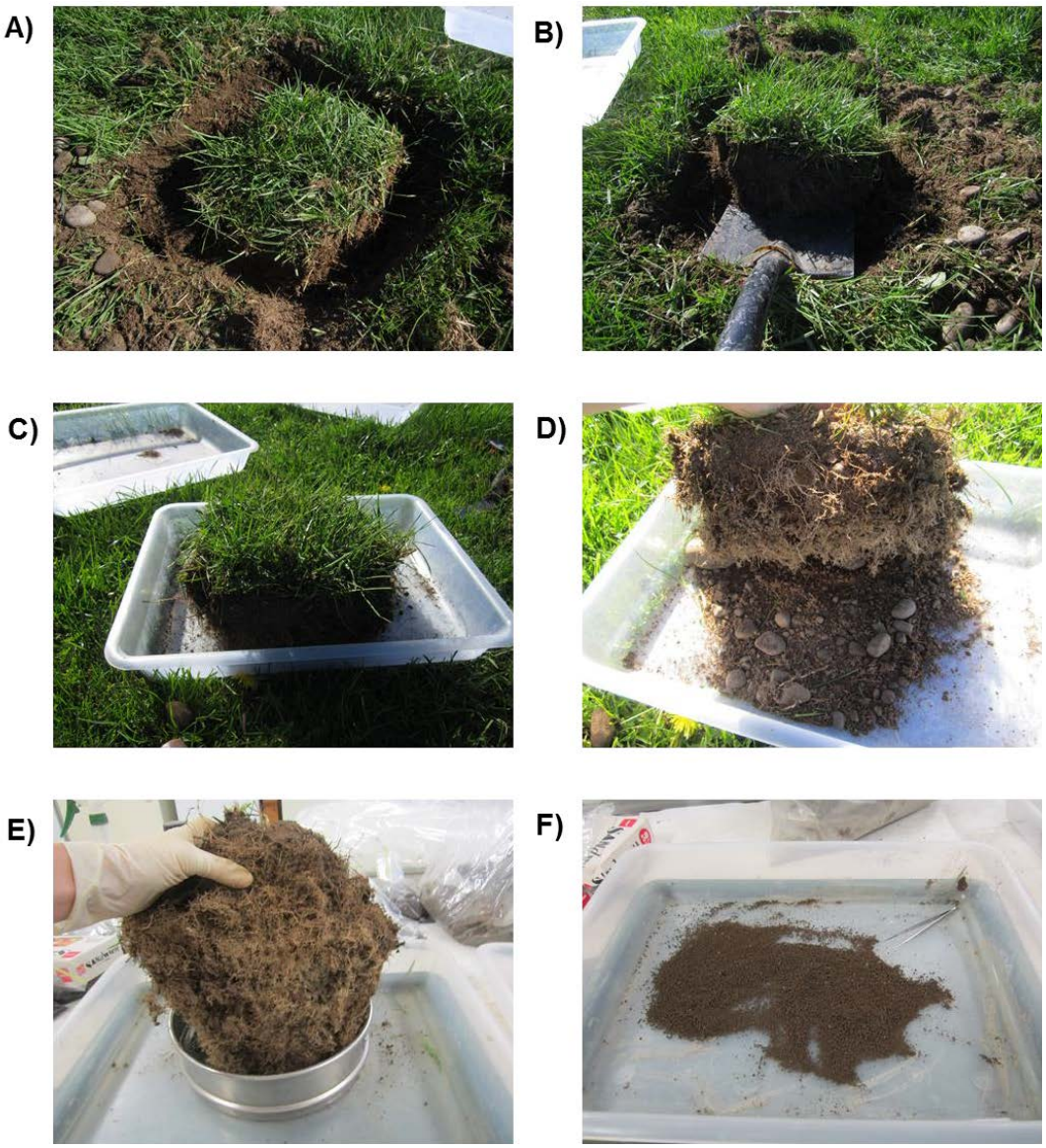


Figure 8.2 Excavation of a pasture slab (A and B) that was placed in the bucket to collect bulk soil (C and D), and the slab was brought back at the lab, where rhizosphere soil was removed from the root mass (E), and visible plant material was removed from both the rhizosphere and bulk soil (F).

8.2.3 Gas Samples

Twenty grams of field moist soil was placed in 125 mL serum bottles (Sigma Aldrich, Part No. 98334, Milwaukee, WI, USA) for incubation at 20°C. Anaerobic conditions were achieved by evacuating bottles for 10 min and flushing with ultra-high purity (99.999%) helium (He) three times. Details of the calibration, sampling procedure, and equipment can be found in Phillips et al. (2015) and a detailed description of the system can be found in Molstad et al. (2007).

The headspace of each serum bottle was sampled every 3 h for 36 h, and concentrations of N_2O, N_2, O_2, and CO_2 were determined on a gas chromatograph (model GC-2010, Shimadzu, Kyoto, Japan) equipped with an electron capture detector for N_2O and CO_2, and a thermal conductivity detector for N_2 and O_2. Oxygen concentrations were measured to ensure conditions in the bottle stayed anaerobic throughout the experiment, and are not reported. Carbon dioxide concentrations were measured as a cross check to make sure there was detectable microbial activity in the soil, and are not reported.

Concentration data for N_2O and N_2 from each sampling location and soil class (rhizosphere vs. bulk) were plotted to show the temporal kinetics of N_2O and N_2 concentrations over time from 36 h incubation (Supplementary Figure S1). Fluxes of N_2O and N_2 were determined using the linear portion of each curve, meaning a different number of samples were used to calculate each flux (Table 8.3). These changes in concentration were used to calculate N_2O fluxes as outlined in section 3.5, with an allowance for N_2O dissolved in the soil water using the Bunsen absorption coefficient (Tiedje 1982).

Table 8.3 The number of data points used for each flux calculation, which was based on the linear portion of the gas concentration curve for each sample. "P" represents the paddock number and "S" represents the site number

	Number of points used for flux calculations	
Sampling location	N_2	N_2O-N
P1-S1-Bulk	2	3
P1-S1-Rhizosphere	2	2
P1-S2-Bulk	6	4
P1-S2- Rhizosphere	6	6
P2-S1-Bulk	7	6
P2-S1- Rhizosphere	7	3
P2-S2-Bulk	5	2
P2-S2- Rhizosphere	4	2
P3-S1-Bulk	4	2
P3-S1- Rhizosphere	5	2
P3-S2-Bulk	5	2
P3-S2- Rhizosphere	3	2

8.2.4 Ancillary Soil Biological, Chemical, and Environmental Sampling

At Lincoln University, two days after the soil collection, denitrification enzyme assays (DEA) were performed to determine denitrification potential, from potential N_2O+N_2 (called DEA-N_2O+N_2),

potential N_2O (called DEA-N_2O) and the potential ratio of $N_2O/(N_2O+N_2)$ (called DEA-$N_2O/(DEA-N_2O+N_2)$) (section 3.5). Inorganic N (NO_3^--N, NH_4^+-N), organic C concentration (cold water carbon, CWC, and hot water carbon, HWC), soil pH, and conductivity (section 3.3) were also measured from each soil class and sampling location.

8.2.5 Data and Statistical Analyses

All data analyses were performed in Minitab (Minitab Inc. version 17 2010). Data were analysed using parametric statistics. When required, data were transformed using a box-cox transform to improve the normality of the distribution (Supplementary Table S1). When data were transformed, conclusions were drawn from the analysis on the transformed scale. The data presented in the tables and figures are untransformed. A critical value of $P = 0.10$ for significance was used.

Table 8.4 Data transformations for statistics using box cox transforms (ʎ)

	Variable (units)	Transform
Gas fluxes	N_2 fluxes (mg-N kg^{-1} h^{-1})	N/A
	N_2O fluxes (mg-N kg^{-1} h^{-1}) (+1)	N/A
	$N_2O:N_2$ (+1)	N/A
Denitrification enzyme assay	DEA-N_2O+N_2	N/A
	DEA-N_2O	N/A
	DEA-$N_2O/(DEA-N_2O+N_2)$	N/A
Soil environmental data	θg (g g^{-1})	ʎ = 2
	NH_4^+-N (µg g^{-1} dry $soil^{-1}$)	ʎ = 5
	NO_3^--N (µg g^{-1} dry $soil^{-1}$)	ʎ = 5
	CWC (µg g^{-1} dry $soil^{-1}$)	N/A
	HWC (µg g^{-1} dry $soil^{-1}$)	N/A
	Soil pH (µg g^{-1} dry $soil^{-1}$)	N/A
	Conductivity (% soluble salt)	N/A

Differences in means between the rhizosphere and bulk soil for N_2O and N_2 gas fluxes, the ratio of $N_2O:N_2$, the DEA-N_2O, DEA-N_2O+N_2, DEA-$N_2O/(DEA-N_2O+N_2)$ and soil chemical data (NO_3^--N, NH_4^+-N, CWC, HWC, pH, and conductivity) were tested using an independent T-test.

A power analysis was completed to determine the number of samples required in order to detect a difference of a given size with a degree of confidence between the bulk and rhizosphere soil classes. This was completed for sample sizes between 3 and 90 (in increments of 3) using the pooled standard deviation from the collected data. The power analysis was run twice for each measured variable (gas fluxes, DEA's, and soil environmental data). The first run was completed

using a beta value (β, probability of making a type II error, or failing to reject the null hypothesis when it is false) of 0.05 and an alpha (α, significance level) of 0.05 (Supplementary Table S 2). The second run was completed using a β value of 0.10 and an α of 0.10 (Supplementary Table S 3).

Data from the rhizosphere and bulk soil were pooled together to test for differences between paddocks using a one-way ANOVA. When required, Tukey's multiple comparison was used as a post-hoc test to determine differences between paddocks.

Linear regression was used to assess the nature and strength of the relationships between gas fluxes, DEA data, and the soil environmental data. Gas fluxes and DEA data were dependent variables, and the soil environment data were independent variables.

8.3 Results

8.3.1 Differences Between Rhizosphere and Bulk Soil

The N_2 and N_2O fluxes tended to be higher, and the ratio of N_2O:N_2 tended to be lower, from the rhizosphere soil compared to the bulk soil. However, there was no significant difference between mean N_2 fluxes (Figure 8.3 a, Table 8.5), mean N_2O fluxes (Figure 8.3 b, Table 8.5), or mean ratios of N_2O:N_2 (Figure 8.3 c, Table 8.5).

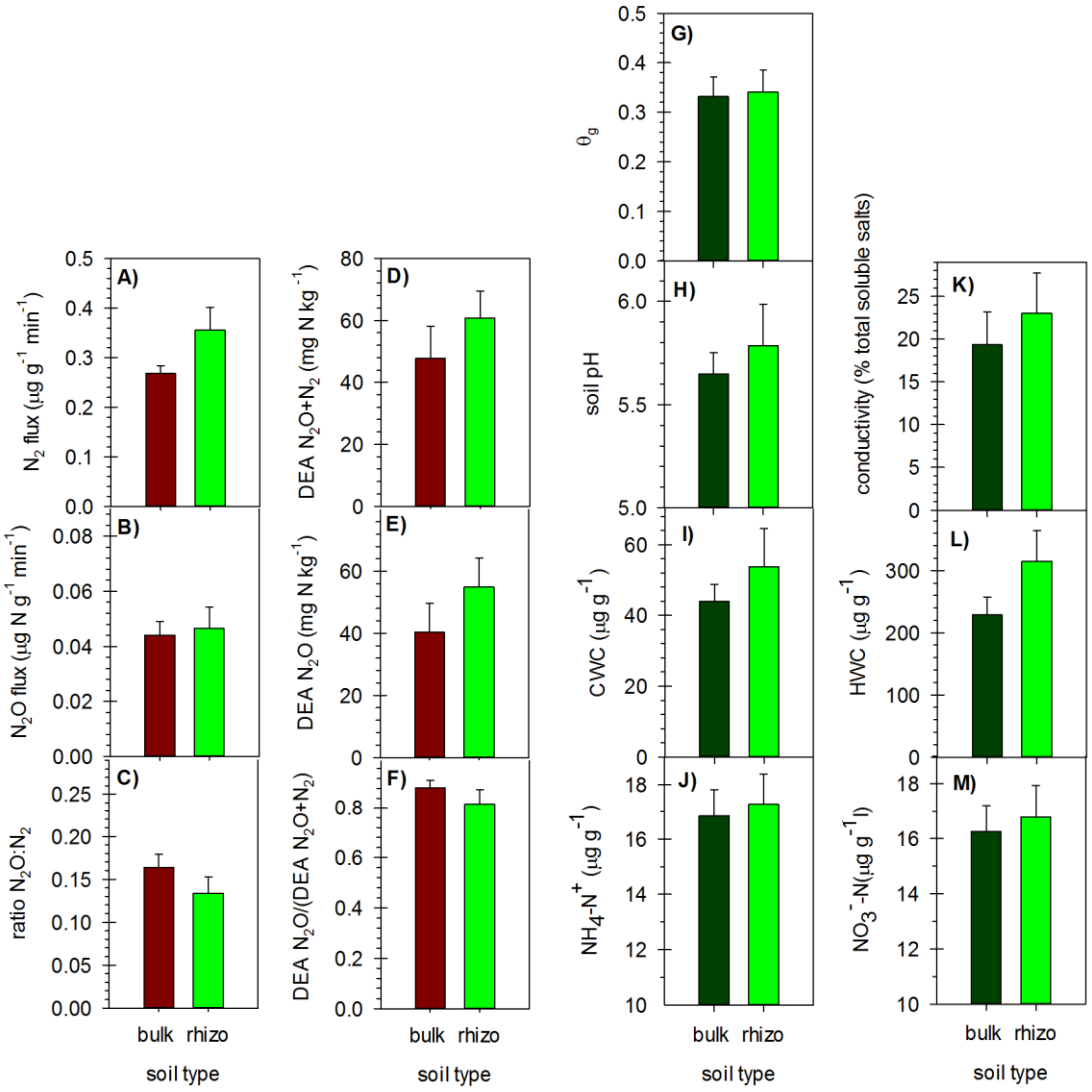


Figure 8.3 The mean and standard error of the mean of bulk and rhizosphere (rhizo) soil A) N_2 fluxes, B) N_2O fluxes, C) ratio of N_2O:N_2 measured from the gas chromatograph, and the mean and standard error of the mean from the denitrification enzyme assays for the D) DEA N_2O+N_2, E) DEA N_2O, and F) the DEA $N_2O/(DEA\text{-}N_2O+N_2)$, G) gravimetric soil moisture, H) soil pH, I) cold water carbon, J) ammonium, L) conductivity, J) hot water carbon, and M) nitrate.

Similarly, the DEA-N_2O+N_2 and DEA-N_2O tended to be higher, and the DEA-N_2O/(DEA-N_2O+N_2) tended to be lower, from the rhizosphere soil compared to the bulk soil, however, there was too much variability in the denitrification potential results to distinguish differences between soil classes. There was no difference between the rhizosphere and bulk soils with regard to DEA-N_2O+N_2 (Figure 8.3 d, Table 8.5), DEA-N_2O (Figure 8.3 e, Table 8.5), or DEA-N_2O/(DEA-N_2O+N_2) (Figure 8.3 f, Table 8.5).

Mean soil environmental and soil chemical variables tended to be higher in the rhizosphere soil compared to the bulk soil but there was no statistical differences between soil classes (Figure 8.3 g to m, Table 8.5).

Table 8.5 Summary statistics for the rhizosphere and bulk soil, and the P-values from the T-test results comparing the rhizosphere and bulk soil variables

Variable (units)	Rhizosphere soil		Bulk soil		P- value
	Mean	±SEM	Mean	±SEM	
N_2 flux (μg N g^{-1} h^{-1})	0.355	0.047	0.268	0.016	0.127
N_2O flux (μg N g^{-1} h^{-1})	0.047	0.008	0.044	0.005	0.798
N_2O:N_2 ratio	0.134	0.019	0.164	0.015	0.249
DEA-N_2O+N_2 (μg N g^{-1} min^{-1})	60.8	8.5	47.7	10.0	0.357
DEA-N_2O (μg N g^{-1} min^{-1})	54.9	9.3	40.3	9.4	0.298
DEA-ratio	0.82	0.06	0.88	0.03	0.351
θg (g g^{-1} dry soil)	0.34	0.05	0.33	0.04	0.797
NH_4^+-N (μg g^{-1} dry soil)	17.29	1.11	16.84	0.98	0.586
NO_3^--N (μg g^{-1} dry soil)	16.78	1.15	16.26	0.92	0.542
CWC (μg g^{-1} dry soil)	53.70	10.90	43.93	4.84	0.524
HWC (μg g^{-1} dry soil)	315.0	49.2	228.8	28.6	0.161
pH	5.79	0.20	5.65	0.10	0.553
Conductivity (% total soluble salts)	23.01	4.70	19.34	3.86	0.681

Based on the power analysis parameters and the pooled standard deviations from the collected data, between 21 and more than 90 samples would be required to detect a difference between the bulk and rhizosphere soil with confidence (Supplementary Table S1. Supplementary Table S2). Interestingly, +90 samples would be needed to detect differences in N_2O fluxes, but 33 samples or less would be needed to detect differences between N_2 fluxes. Likewise, all soil chemical factors need +90 samples, except HWC, which needed 39 or less (Table 8.6).

Table 8.6 The mean and standard error of the mean (SEM, where n=12 for the total, and n=4 for Paddocks 1, 2 and 3) for the denitrification enzyme assay data, the fluxes from the chromatograph, and the environmental data. This is pooled data from both the rhizosphere and bulk soil.

	Paddock						One-way ANOVA
	1		3		2		
Variable (Units)	Mean	±SEM	Mean	±SEM	Mean	±SEM	P value
N_2 flux (μg N g^{-1} h^{-1})	0.2988	0.0727	0.2924	0.0346	0.344	0.0319	0.736
N_2O flux (μg N g^{-1} h^{-1})	0.0322	0.0062	0.0471	0.0044	0.0567	0.0075	0.056
N_2O:N_2 ratio	0.1111	0.0058	0.1705	0.0280	0.1648	0.0128	0.085
DEA-N_2O+N_2 (μg N g^{-1} min^{-1})	45.80	10.70	39.74	8.42	77.26	6.69	0.030
DEA-N_2O (mg N kg^{-1} h^{-1})	35.30	8.61	35.43	7.06	72.14	8.12	0.014
DEA-ratio	0.77	0.04	0.86	0.07	0.92	0.04	0.163
θg (g g^{-1} dry soil)	0.26	0.08	0.37	0.01	0.38	0.02	0.219
NH_4^+-N (μg g^{-1} dry soil)	15.27	1.86	17.70	0.20	18.23	0.60	0.312
NO_3^-N (μg g^{-1} dry soil)	14.99	1.97	16.60	0.181	17.99	0.31	0.316
CWC (μg g^{-1} dry soil)	47.1	18.3	44.3	0.7	55.1	4.8	0.776
HWC (μg g^{-1} dry soil)	200.4	71.1	290.4	16.6	325.0	43.2	0.232
soil pH	5.70	0.31	5.59	0.02	5.86	0.14	0.643
Conductivity (total soluble salts, %)	24.32	3.97	13.27	1.04	25.93	6.88	0.165

8.3.2 Differences Between Paddocks

A comparison of means between the paddocks showed some statistical difference between the paddocks for N_2 and N_2O fluxes. Dinitrogen fluxes were highest in paddock 2, and were higher in paddock 2 and 3 compared to paddock 1. Mean N_2 fluxes were similar in paddock 1 and 3, but more variability was observed in the data from paddock 1 compared to paddock 3 (Figure 8.4 a, Table 8.6). Nitrous oxide fluxes were lowest in paddock 1, and were highest in paddock 2, and N_2O fluxes were significantly higher in paddock 1 compared to paddock 2 (Figure 8.4 b, Table 8.6). The ratio of N_2O:N_2 reflected a different trend. The ratio of N_2O:N_2 was highest in paddock 3 and lowest in paddock 1 but there was no significant difference between paddocks (Figure 8.4 c, Table 8.6).

Generally, there was higher denitrification potential noted in paddock 2 compared to the other paddocks. The DEA-N_2O+N_2 was 49% higher in paddock 2 compared to paddock 3. There was no difference between DEA-N_2O+N_2 between paddocks 1 and 2, but DEA-N_2O was 104% higher in paddock 2 compared to paddock 1, and 51% higher in paddock 2 compared to paddock 3 (Figure 8.4 a-b, Table 8.6). The ratio of DEA-N_2O/(DEA-N_2O+N_2) was lowest in paddock 1, and the highest in paddock 3 (Figure 8.4 c, Table 8.6).

A comparison of means between the paddocks failed to note any statistical difference between the paddocks for soil chemical factors. Soil moisture and HWC were lowest in paddock 1, and highest in paddock 2 (Figure 8.4). The soil pH, conductivity, and CWC showed similar trends, with the lowest observed in paddock 3, and the highest observed in paddock 2 (Figure 8.4 h, i, and j, Table 8.6). In contrast to this, NH_4^+-N and NO_3^--N were highest in paddock 3 (Figure 8.4 l and m, Table 8.6).

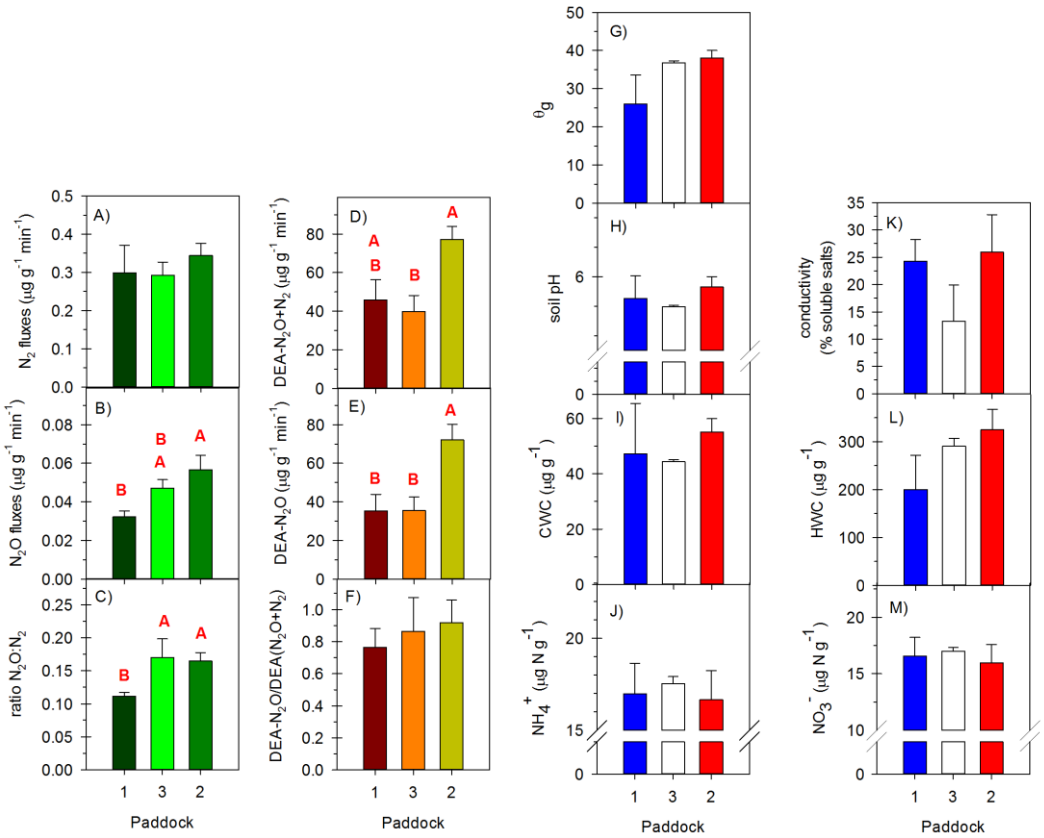


Figure 8.4 The mean and standard error of the mean for paddocks 1, 2 and 3 for A) N_2 fluxes, B) N_2O fluxes, C) ratio of $N_2O/(N_2O+N_2)$ measured from the gas chromatograph, and the mean and standard error of the mean for the denitrification enzyme assays for the D) DEA N_2O+N_2, E) DEA N_2O, and F) the DEA-N_2O/(DEA-N_2O+N_2), G) gravimetric soil moisture, H) soil pH, I) cold water carbon, J) ammonium, K) conductivity, L) hot water carbon, and M) nitrate. Means that do not share a letter are significantly different.

8.3.3 Explaining Variability in Denitrification Potential and Gas Fluxes

Variability in N_2 fluxes was positively related to CWC and HWC, as well as NO_3^--N and NH_4^+-N (Supplementary Figure S1, Table 8.7). Variability in N_2O fluxes was strongly and positively related to CWC, and was also positively related to HWC, pH, and θg (Supplementary Figure S2, Table 8.7). The ratio of N_2O:N_2 was not related to any of the soil environmental factors (Supplementary Figure S3, Table 8.7).

Variability in DEA-N_2O+N_2 was best explained by NO_3^- and pH, but was also related to all of the other environmental factors (Supplementary Figure S5, Table 8.7). Variability in DEA-N_2O was best explained by NO_3^--N and HWC, but was also related to all of the other environmental factors except NH_4^+-N (Supplementary Figure S4, Table 8.7). Variability in the DEA N_2O/(DEA-N_2O+N_2) was not relate to any related environmental factors (Supplementary Figure S7, Table 8.7).

Table 8.7 Linear regression results between gas fluxes and environmental variables with all data pooled together

	N_2 flux (µg N g^{-1} h^{-1})		N_2O flux (µg N g^{-1} h^{-1})		N_2O:N_2		DEA-N_2O+N_2 (µg N g^{-1} min^{-1})		DEA-N_2O (µg N g^{-1} min^{-1})		DEA-ratio (DEA-N_2O/DEA-N_2O+N_2)	
Variable	P-value	r^2	P-value	r^2	P-value	r^2	P-value	r^2	P-value	r^2	P-value	r^2
θg	0.039	0.36	0.070	0.29	0.460	0.06	0.076	0.28	0.093	0.26	0.473	0.05
NH_4^+-N	0.033	0.38	0.053	0.32	0.388	0.08	0.084	0.27	0.105	0.24	0.448	0.06
NO_3^--N	0.001	0.72	0.018	0.44	0.570	0.03	0.005	0.57	0.007	0.54	0.696	0.02
CWC	0.000	0.75	0.039	0.36	0.939	0.00	0.042	0.35	0.049	0.33	0.953	0.00
HWC	0.003	0.61	0.008	0.53	0.592	0.03	0.031	0.39	0.016	0.46	0.491	0.05
pH	0.002	0.63	0.030	0.39	0.963	0.00	0.023	0.42	0.028	0.40	0.887	0.00
conductivity	0.101	0.25	0.166	0.18	0.828	0.00	0.044	0.35	0.036	0.37	1.00	0.00

8.4 Discussion

The N_2O and N_2 fluxes observed in this study were lower than those observed by Phillips et al. (2015). They reported fluxes ranging from 0.43 to 16.3 μg g^{-1} h^{-1} N_2O, and 0.14 to 0.73 μg g^{-1} h^{-1} N_2, depending on the temperature, from a Manawatu silt-loam soil sown with ryegrass and clover, and grazed by sheep. Their higher fluxes may be attributed to a number of factors, including the more frequent gas sampling regime, as well as differences in soil type and land use history.

The results from the current study failed to support the original hypothesis that N_2O and N_2 fluxes would be higher in the rhizosphere soil compared to bulk soil. It was expected that these higher N_2O and N_2 fluxes in the rhizosphere would be supported by higher inorganic N, organic C, and differences in soil pH and conductivity, and higher denitrification potential (Table 8.1), which is indicative of greater biological denitrification capability in the soil. While means of these factors tended to be higher in the rhizosphere compared to the bulk soil, there was not enough of a difference between the soil classes for there to be statistical differences. There are a number of potential reasons for the lack of difference between the bulk and rhizosphere soil in this study including ineffective separation of the soil classes, too few samples, and spatial variability.

The similarities between bulk and rhizosphere soil for all measured factors could be due to ineffective separation of rhizosphere and bulk soil. This could mean 1) a more precise method for separating soil is required, or 2) the fine root mass of the pasture plant created a rhizosphere that affected all soil within the top 100 mm of the rhizosphere to some degree (Figure 8.2). Deciphering this is work for future studies. Studies that have used field collected soils to isolate differences in bacterial communities between rhizosphere and bulk soil have used "plant shaking" similar to the method used in this study to separate bulk soil from the rhizosphere soil from potato, strawberry, and oilseed rape plants (Smalla et al. 2001). These plants tend to have less tangled and less fibrous roots compared to the root mass produced by the pasture in the current study. It may be that the root structure (Figure 8.2 d-e) of the pasture species inherently makes separation of rhizosphere and bulk soil for pasture plants difficult. Soil characteristics such as higher soil moisture, higher soil clay content, and high compaction will encourage aggregation which could make soil separation more difficult. These factors were probably not an issue in this study as the soil was dry (~35% gravimetric soil moisture), with a low clay content and low bulk density (section 4.3.1). However, since the rhizosphere has no definitive boundary, there was no way to know whether the rhizosphere soil was contaminated with bulk soil, and vice versa.

The power analysis results suggest that more samples would have helped give confidence to the results. Based on the parameters used for the power analysis, often more than 90 samples would be required for there to be confidence in the statistical results between the rhizosphere and bulk soil. The power analysis is based on the pooled standard deviation of the collected data. The standard deviation inherently encompasses the variability observed between paddocks. If more samples were collected from within the individual paddocks, and the power analysis was re-run using a standard deviation derived from within the individual paddocks, it may show that less samples are needed to detect the difference between the bulk and the rhizosphere soil within each individual paddock with confidence.

The inability to detect a difference between the rhizosphere and bulk soil may also be attributed to high variability inherent in the natural environment, which was likely confounded by variability induced by grazing, land management, or variation in soil properties. While often not statistically significant, there were differences between paddocks and trends that warrant discussion. Specifically, there were relationships between gas fluxes, and DEA, and NO_3^- and C concentrations (Table 8.7). The variability between the paddocks may be linked to grazing rotation. Compaction from trampling has been shown to increase N_2O fluxes (Ruser et al. 1998, Bhandral et al. 2007, Beare et al. 2009) and N_2 fluxes (Ruser et al. 2006). However, at D_P/D_O of 0.006 or less, reduction of N_2O to N_2 occurs (Balaine et al. 2013, Harrison-Kirk et al. 2015), and a history of O_2 depletion has been shown to prime the N_2OR pathway (Uchida et al. 2014). This means that compaction-induced reduction in soil O_2 concentrations could have promoted N_2OR, resulting in the observed differences in N_2O/N_2 ratio between paddocks. This study did not measure D_P/D_O during sampling collection, and the soils were sieved for analysis, so this hypothesis could not be explored in this study. The link between D_P/D_O and trampling, and how this influences the ensuing N_2O and N_2 fluxes from the soil from different paddocks, should be explored in future studies.

Variability in soil nutrients between paddocks influenced N_2O and N_2 flux dynamics. Urine patches may cover ≈ 20% of a grazed pasture, annually (Moir et al., 2011), and a history of urine deposition can change microbial community size and functioning, thus altering soil N cycling (Wakelin et al., 2013). The potential of a combination of inter- vs. intra- urine patch sampling, along with potentially sampling areas of historical urine patches of different ages, may have contributed to variability. Fertilizer history may influence the N_2O and N_2 dynamics and may play an important role in explaining spatial variability of fluxes between paddocks. Nitrogen additions can prime the soil microbial community to mobilize N within the pre-existing soil organic matter (Kuzyakov et al.

2000). This enhances soil native N mobilization, and can result in higher N_2O emissions derived from the soil N pool (Di and Cameron 2008). Likewise, grazing has been suggested to increase C availability in the rhizosphere as a result of a pasture being grazed. This increase in C may encourage greater denitrification potential and increase N_2OR if the higher C availability leads to increased C respiration, and therefore a decrease in O_2 (Morley and Baggs 2010). While there was the high variability in HWC (ranging from 100 to 400 μg g^{-1}) and NO_3^- (ranging from ≈12 to ≈20 μg g^{-1}) observed in this study, and there were significant relationships between these factors and N_2 fluxes, N_2O fluxes, DEA-N_2O, and DEA-N_2O+N_2 (Table 8.7). The effect of fertilizer and grazing on spatial variability of fluxes and denitrification potential cannot be confirmed because sampling for this study only occurred at one point in time. The between paddock variability may be inherent natural variability, and may have nothing to do with the current land management practices. These factors should be considered in future sampling campaigns.

The regression analysis noted relationships between N_2 fluxes, N_2O fluxes, DEA-N_2O, and DEA-N_2O+N_2, and environmental factors (Table 8.7). Clearer results were expected, given that the proximal controllers for denitrification are well defined (Firestone et al. 1980, Wallenstein et al. 2006). However, the magnitude of variability in many of the environmental factors was actually small, and in some cases, biased by one or two points (Supplementary Figure S2 to S7). For example, a positive relationship between pH and N_2O, and a negative relationship between pH and N_2 fluxes, was observed, but the relationship was not significant. While pH is a regulator of N_2OR (Firestone et al. 1980, Knowles 1982, Stevens et al. 1998, Wallenstein et al. 2006), this magnitude of variability is small and is inherent in field studies where the environmental conditions are not manipulated.

8.5 Conclusions

There were no differences in N_2O or N_2 fluxes observed between rhizosphere and bulk soil classes. This may be because the technique used to separate the soil was not able to effectively separate the soil classes. It may also be due to the spatial variably within the paddocks from a history of urine deposition or fertilizer application, or variability between the paddocks either from natural variation or management-induced variation, overrode the more subtle differences in soil classes. More research is needed to assess the differences in N_2OR capabilities between rhizosphere and bulk soil. Future research that does not composite samples should include more samples to improve confidence in statistical analysis.

Supplementary Data

The supplementary tables shows the transformations used for the statistics (Supplementary Table S1), and detailed results from the power analyses (Supplementary Table S2 and Supplementary Table S3). The supplementary figures show kinetics of the N_2O fluxes measured for each incubation for each soil class and site (Supplementary Figure S1), and scatter plots with the N_2 fluxes, N_2O fluxes, N_2O:N_2 ratios, DEA-N_2O+N_2, DEA-N_2O, and DEA-N_2O/(DEA-N_2O+N_2) plotted against the soil environmental variables (Supplementary Figure S2 to Supplementary Figure S7).

8.6 References

Balaine, N., T. Clough, M. Beare, S. Thomas, E. Meenken, and J. Ross. 2013. Changes in Relative Gas Diffusivity Explain Soil Nitrous Oxide Flux Dynamics. Soil Science Society of America Journal 77:1496-1505.

Beare, M. H., E. G. Gregorich, and P. St-Georges. 2009. Compaction effects on CO_2 and N_2O production during drying and rewetting of soil. Soil Biology and Biochemistry 41:611-621.

Bhandral, R., S. Saggar, N. Bolan, and M. Hedley. 2007. Transformation of nitrogen and nitrous oxide emission from grassland soils as affected by compaction. Soil and Tillage Research 94:482-492.

Cheng, W., D. Johnson, and S. Fu. 2003. Rhizosphere effects of decomposition - controls of plant species, phenology and fertilization. Soil Science Society of America Journal 67:1418-1427.

Di, H. J., and K. Cameron. 2008. Sources of nitrous oxide from ^{15}N-labelled animal urine and urea fertiliser with and without a nitrification inhibitor, dicyandiamide (DCD). Australian Journal of Soil Research 46:76-82.

Firestone, M. K., R. B. Firestone, and J. M. Tiedje. 1980. Nitrous oxide from soil denitrification: Factors controlling its biological production. Science 208:749-751.

Foster, R. C. 1988. Microenvironments of soil microorganisms. Biology and Fertility of Soils 6:189-203.

Garbeva, P., J. A. van Veen, and J. D. van Elsas. 2004. Microbial diversity in soil: selection microbial populations by plant and soil type and implications for disease suppressiveness. Annual Review of Phytopathology 42:243-270.

Hall, J. M., E. Paterson, and K. Killham. 1998. The effect of elevated CO_2 concentration and soil pH on the relationship between plant growth and rhizosphere denitrification potential. Global Change Biology 4:209-216.

Harrison-Kirk, T., S. Thomas, T. Clough, M. Beare, T. van der Weerden, and E. Meenken. 2015. Compaction influences N_2O and N_2 emissions from ^{15}N-labeled synthetic urine in wet soils during successive saturation/drainage cycles. Soil Biology and Biochemistry 88:178-188.

Hinsinger, P., G. Gobran, P. Gregory, and W. Wenzel. 2005. Rhizosphere ge ometry and heterogeneity arising from root-mediated physical and chemical processes. The New Phytologist 168:293-303.

Højberg, O., S. Binnerup, and J. Sørensen. 1996. Potential rates of ammonium oxidation, nitrite oxidation, nitrate reduction and denitrification in the young barley rhizosphere. Soil Biology and Biochemistry 28:47-54.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485-1498.

Lynch, J. M., and J. M. Whipps. 1990. Substrate flow in the rhizosphere. Plant and Soil 129:1-10.

Mezzari, M., Z. Daniela Mara Hoffmann, H. Corseuil, and A. Nogueira. 2011. Potential of grasses and rhizosphere bacteria for bioremediation of diesel-contaminated soils. Revista Brasileira de Ciência do Solo 35:2227-2236.

Minitab Inc. version 17. 2010. Minitab Statistical Software [Computer software]. State College, PA.

Moir, J. L., K. C. Cameron, H. J. Di, and U. Fertsak. 2011. The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. The Journal of Agricultural Science 149:473-485.

Molstad, L., P. Dörsch, and L. Bakken. 2007. Robotized incubation system for monitoring gases (O_2, NO, N_2O N_2) in denitrifying cultures. Journal of Microbiological Methods 71:202-211.

Morley, N., and E. M. Baggs. 2010. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biology and Biochemistry 42:1864-1871.

Nye, P. H. 1981. Changes of pH across the rhizosphere induced by roots. Plant and Soil 61:7-26.

Philippot, L., S. Hallin, G. Börjesson, and E. M. Baggs. 2009. Biochemical cycling in the rhizosphere having an impact on global change. Plant and Soil 321:61-80.

Phillips, R. L., A. M. S. McMillan, T. Palmada, J. Dando, and D. Giltrap. 2015. Temperature effects on N_2O and N_2 denitrification end-products for a New Zealand pasture soil. New Zealand Journal of Agricultural Research 58:89-95.

Prendergast-Miller, M. T., M. Duvall, and S. P. Sohi. 2014. Biochar-root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability. European Journal of Soil Science 65:173-185.

Rouatt, J. W., H. Katznelson, and T. M. B. Payne. 1960. Statistical evaluation of the rhizosphere effect. Soil Science Society of America Proceedings 24:271-273.

Rovira, A. D. 1965. Interactions Between Plant Roots and Soil Microorganisms. Annual Review of Microbiology 19:241-266.

Ruser, R., H. Flessa, R. Russow, G. Schmidt, F. Buegger, and J. C. Munch. 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting. Soil Biology and Biochemistry 38:263-274.

Ruser, R., R. Schilling, H. Steindl, H. Flessa, and F. Beese. 1998. Soil Compaction and Fertilization Effects on Nitrous Oxide and Methane Fluxes in Potato Fields. Soil Science Society of America Journal 62:1587-1595.

Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, and G. Berg. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology 67:4742-4751.

Sørensen, J. 1997. The rhizosphere as a habitat for soil microorganisms. Pages 21-45 in J. D. Elsas, van, J. T. Trevors, and E. M. H. Wellington, editors. Modern Soil Microbiology.

Stevens, R. J., R. J. Laughlin, and J. P. Malone. 1998. Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. Soil Biology and Biochemistry 30:1119-1126.

Tiedje, J. M. 1982. Denitrification. Agronomy Monograph No. 9.

Uchida, Y., Y. Wang, H. Akiyama, Y. Nakajima, and M. Hayatsu. 2014. Expression of denitrification genes in response to a waterlogging event in a Fluvisol and its relationship with large nitrous oxide pulses. FEMS Microbiology Ecology 88:407-423.

S.S. Wakelin, T.J. Clough, E.M. Gerard, M. O'Callaghan. 2013. Impact of short-interval, repeat application of dicyandiamide on soil N transformation in urine patches. Agriculture, Ecosystems and Environment 167:60–70.

Wallenstein, M., D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications 16:2143-2152.

Zhu, B., J. Gutknecht, D. Herman, D. Keck, M. Firestone, and W. Cheng. 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology and Biochemistry 76:183-192.

8.7 Supplementary Data

Supplementary Table S1 The number of samples required to provide a meaningful statistical comparison for all measured variables.

		power analysis parameters	
		β = 0.05 α = 0.05	β = 0.10 α = 0.10
Variable (Units)	pooled stdev	# samples needed	
N_2 flux (μg N g^{-1} h^{-1})	0.0935	33	21
N_2O flux (μg N g^{-1} h^{-1})	0.01530	+90	+90
DEA-N_2O+N_2 (μg N g^{-1} min^{-1})	23.35	84	57
DEA-N_2O (μg N g^{-1} min^{-1})	23.13	69	45
θg (g g^{-1})	0.10	+90	+90
NH_4^+-N (μg g^{-1})	2.46	+90	+90
NO_3^-N (μg g^{-1})	2.45	+90	+90
CWC (μg g^{-1})	20.30	+90	+90
HWC (μg g^{-1})	104.2	39	27
pH	0.38	+90	+90
conductivity (% soluble salt)	10.22	+90	+90

Supplementary Table S2 Power analysis results for a significance level (alpha) of 0.05 and the probably of making a type II error, or failing to reject the null hypothesis when it is false (beta) of 0.05 and based on the pool standard deviation from the collected data for each measured variable. The actual difference shows the absolute difference between the bulk and rhizosphere soil in the experiment, and the difference required between the two factors, for each permutation of subsamples, to determine significance.

	N_2 flux (μg N g^{-1} h^{-1})	N_2O flux (μg N g^{-1} h^{-1})	N_2O:N_2	DEA-N_2O+N_2 (μg N g^{-1} min^{-1})	DEA-N_2O (μg N g^{-1} min^{-1})	DEA-Ratio	θg (g g^{-1})	NH_4^+-N (μg g^{-1})	NO_3^--N (μg g^{-1})	CWC (μg g^{-1})	HWC (μg g^{-1})	pH	conductivity (% soluble salt)
pooled standard deviation	0.09	0.02	0.04	23.35	23.13	0.11	0.10	2.46	2.45	20.30	104.20	0.38	10.22
actual difference	0.087	0.003	-0.03	13.1	14.6	-0.066	0.0092	0.45	0.52	9.77	86.2	0.14	3.67
sample size	Difference required to detect a significant difference between the bulk and rhizosphere soil												
3	0.376	0.062	0.173	93.95	93.07	0.46	0.40	9.89	9.86	81.68	419.27	1.51	41.12
6	0.217	0.035	0.100	54.09	53.58	0.26	0.23	5.69	5.68	47.03	241.40	0.87	23.68
9	0.169	0.028	0.078	42.31	41.92	0.21	0.18	4.45	4.44	36.79	188.83	0.68	18.52
12	0.144	0.024	0.066	35.98	35.64	0.18	0.15	3.79	3.78	31.28	160.55	0.58	15.75
15	0.128	0.021	0.059	31.85	31.55	0.16	0.13	3.35	3.34	27.69	142.14	0.51	13.94
18	0.116	0.019	0.053	28.89	28.62	0.14	0.12	3.04	3.03	25.11	128.92	0.46	12.64
21	0.107	0.017	0.049	26.63	26.37	0.13	0.11	2.80	2.79	23.15	118.82	0.43	11.65
24	0.099	0.016	0.046	24.82	24.59	0.12	0.11	2.61	2.61	21.58	110.78	0.40	10.87
27	0.093	0.015	0.043	23.35	23.13	0.11	0.10	2.46	2.45	20.30	104.18	0.37	10.22
30	0.089	0.014	0.041	22.10	21.89	0.11	0.09	2.33	2.32	19.22	98.64	0.35	9.67
33	0.084	0.014	0.039	21.04	20.84	0.10	0.09	2.21	2.21	18.29	93.89	0.34	9.21

36	0.081	0.013	0.037	20.12	19.93	0.10	0.09	2.12	2.11	17.49	89.78	0.32	8.81
39	0.077	0.013	0.036	19.31	19.13	0.09	0.08	2.03	2.03	16.79	86.16	0.31	8.45
42	0.074	0.012	0.034	18.59	18.41	0.09	0.08	1.96	1.95	16.16	82.95	0.30	8.14
45	0.072	0.012	0.033	17.94	17.77	0.09	0.08	1.89	1.88	15.60	80.07	0.29	7.85
48	0.070	0.011	0.032	17.36	17.20	0.08	0.07	1.83	1.82	15.09	77.47	0.28	7.60
51	0.067	0.011	0.031	16.83	16.67	0.08	0.07	1.77	1.77	14.63	75.11	0.27	7.37
54	0.065	0.011	0.030	16.35	16.19	0.08	0.07	1.72	1.72	14.21	72.95	0.26	7.16
57	0.064	0.010	0.029	15.90	15.75	0.08	0.07	1.67	1.67	13.83	70.97	0.26	6.96
60	0.062	0.010	0.029	15.49	15.35	0.08	0.07	1.63	1.63	13.47	69.15	0.25	6.78
63	0.061	0.010	0.028	15.12	14.97	0.07	0.06	1.59	1.59	13.14	67.45	0.24	6.62
66	0.059	0.010	0.027	14.76	14.62	0.07	0.06	1.55	1.55	12.83	65.88	0.24	6.46
69	0.058	0.009	0.027	14.43	14.30	0.07	0.06	1.52	1.51	12.55	64.41	0.23	6.32
72	0.057	0.009	0.026	14.12	13.99	0.07	0.06	1.49	1.48	12.28	63.03	0.23	6.18
75	0.055	0.009	0.025	13.84	13.71	0.07	0.06	1.46	1.45	12.03	61.74	0.22	6.06
78	0.054	0.009	0.025	13.56	13.44	0.07	0.06	1.43	1.42	11.79	60.53	0.22	5.94
81	0.053	0.009	0.025	13.31	13.18	0.07	0.06	1.40	1.40	11.57	59.38	0.21	5.82
84	0.052	0.009	0.024	13.06	12.94	0.06	0.06	1.37	1.37	11.36	58.30	0.21	5.72
87	0.051	0.008	0.024	12.83	12.71	0.06	0.05	1.35	1.35	11.16	57.27	0.21	5.62
90	0.051	0.008	0.023	12.62	12.50	0.06	0.05	1.33	1.32	10.97	56.30	0.20	5.52

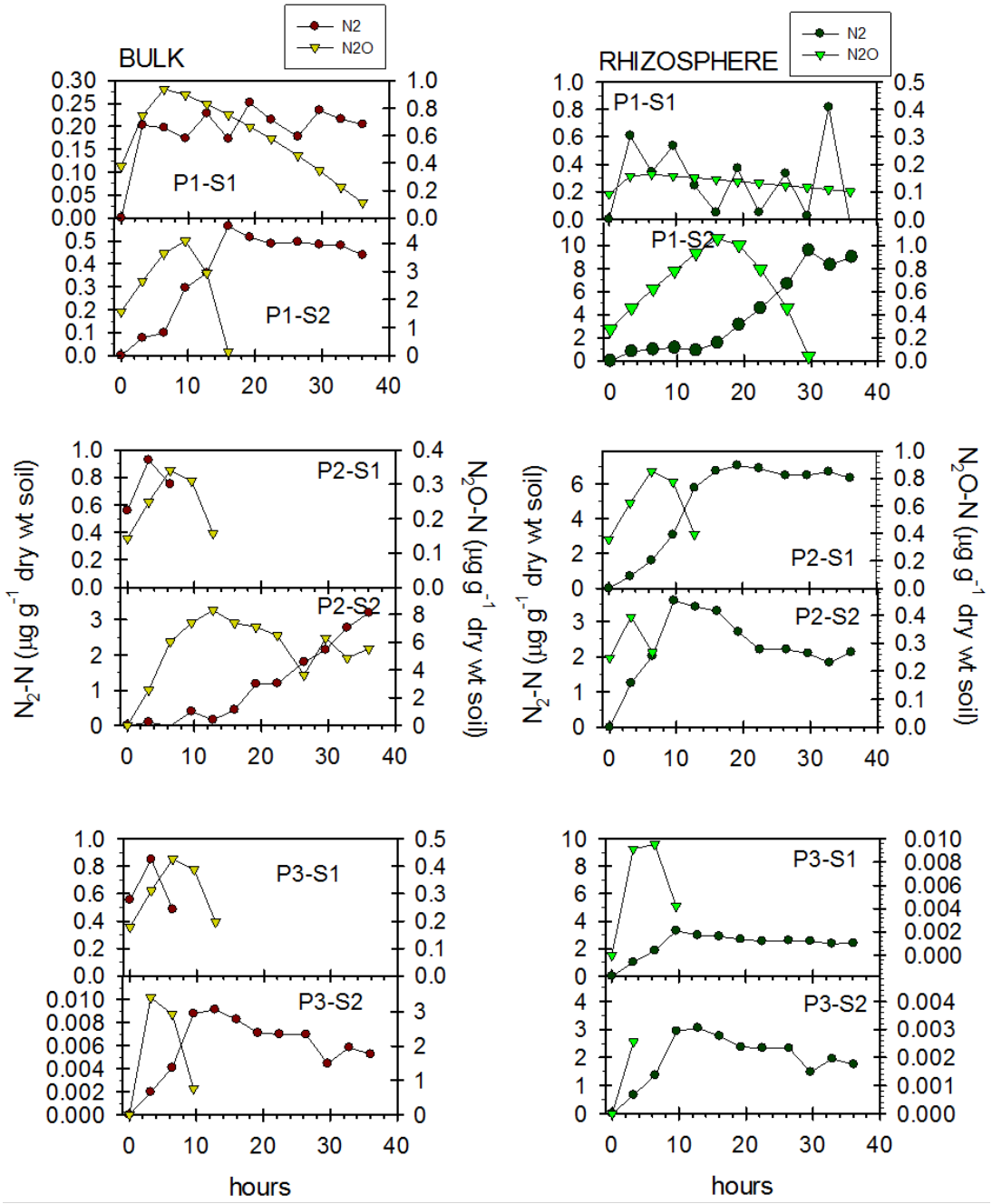
Power, 0.95 = 1-β, where β is the probability of making a type II error (failing to reject the null hypothesis when it is false). β is assume d here to be 0.05.

Supplementary Table S3 Power analysis results for a significance level (alpha) of 0.10 and the probably of making a type II error, or failing to reject the null hypothesis when it is false (beta) of 0.10 and based on the pool standard deviation from the collected data for each measured variable. The actual difference shows the absolute difference between the bulk and rhizosphere soil in the experiment, and the difference required between the two factors, for each permutation of subsamples, to determine significance.

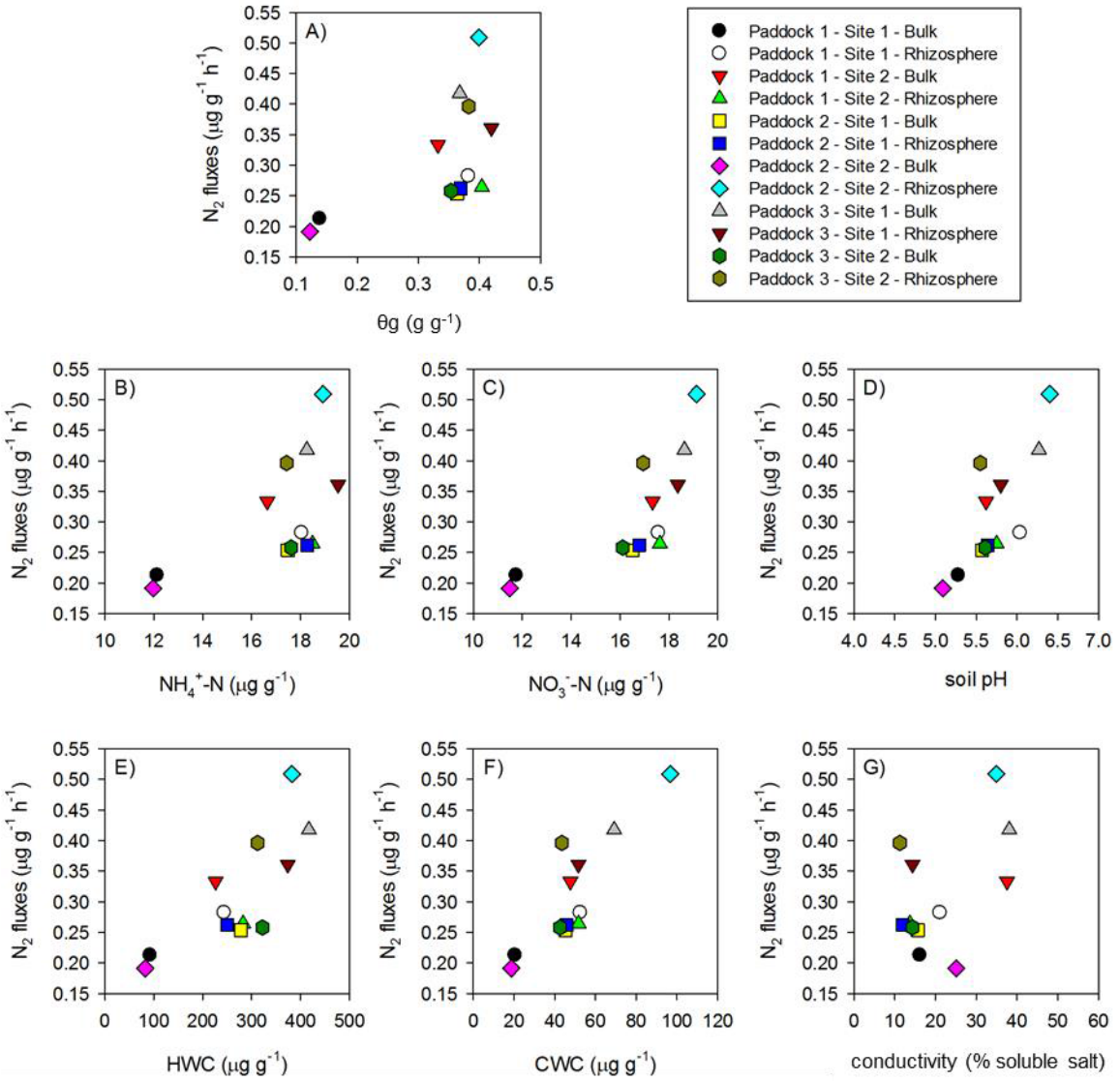
	N_2 flux (μg N g^{-1} h^{-1})	N_2O flux (μg N g^{-1} h^{-1})	N_2O:N_2	DEA-N_2O+N_2 (μg N g^{-1} min^{-1})	DEA-N_2O (μg N g^{-1} min^{-1})	DEA-Ratio	θg (g g^{-1})	NH_4^+-N (μg g^{-1})	NO_3^--N (μg g^{-1})	CWC (μg g^{-1})	HWC (μg g^{-1})	pH	conductivity (% soluble salt)
pooled STDEV	0.09	0.02	0.04	23.35	23.13	0.11	0.10	2.46	2.45	20.30	104.20	0.38	10.22
actual difference	0.087	0.003	-0.03	13.1	14.6	-0.066	0.0092	0.45	0.52	9.77	86.2	0.14	3.67
sample size	Difference required to detect a significant difference between the bulk and rhizosphere soil												
3	0.275	0.045	0.126	68.63	67.99	0.34	0.29	7.22	7.20	59.67	306.28	1.10	30.04
6	0.170	0.028	0.078	42.46	42.06	0.21	0.18	4.47	4.46	36.91	189.47	0.68	18.58
9	0.135	0.022	0.062	33.68	33.36	0.16	0.14	3.54	3.54	29.28	150.29	0.54	14.74
12	0.115	0.019	0.053	28.80	28.53	0.14	0.12	3.03	3.02	25.04	128.53	0.46	12.61
15	0.102	0.017	0.047	25.58	25.34	0.13	0.11	2.69	2.69	22.24	114.15	0.41	11.20
18	0.093	0.015	0.043	23.25	23.03	0.11	0.10	2.45	2.44	20.21	103.74	0.37	10.17
21	0.086	0.014	0.040	21.45	21.25	0.10	0.09	2.26	2.25	18.65	95.74	0.34	9.39
24	0.080	0.013	0.037	20.02	19.83	0.10	0.08	2.11	2.10	17.41	89.35	0.32	8.76
27	0.075	0.012	0.035	18.84	18.67	0.09	0.08	1.98	1.98	16.38	84.10	0.30	8.25
30	0.071	0.012	0.033	17.85	17.68	0.09	0.08	1.88	1.87	15.52	79.67	0.29	7.81

33	0.068	0.011	0.031	17.00	16.84	0.08	0.07	1.79	1.78	14.78	75.88	0.27	7.44
36	0.065	0.011	0.030	16.26	16.11	0.08	0.07	1.71	1.71	14.14	72.58	0.26	7.12
39	0.063	0.010	0.029	15.61	15.47	0.08	0.07	1.64	1.64	13.57	69.68	0.25	6.83
42	0.060	0.010	0.028	15.04	14.89	0.07	0.06	1.58	1.58	13.07	67.10	0.24	6.58
45	0.058	0.010	0.027	14.52	14.38	0.07	0.06	1.53	1.52	12.62	64.79	0.23	6.35
48	0.056	0.009	0.026	14.05	13.92	0.07	0.06	1.48	1.47	12.21	62.70	0.23	6.15
51	0.055	0.009	0.025	13.62	13.50	0.07	0.06	1.43	1.43	11.84	60.80	0.22	5.96
54	0.053	0.009	0.024	13.24	13.11	0.06	0.06	1.39	1.39	11.51	59.06	0.21	5.79
57	0.052	0.008	0.024	12.88	12.76	0.06	0.05	1.36	1.35	11.20	57.47	0.21	5.64
60	0.050	0.008	0.023	12.55	12.43	0.06	0.05	1.32	1.32	10.91	56.00	0.20	5.49
63	0.049	0.008	0.023	12.24	12.13	0.06	0.05	1.29	1.29	10.64	54.63	0.20	5.36
66	0.048	0.008	0.022	11.96	11.84	0.06	0.05	1.26	1.26	10.40	53.36	0.19	5.23
69	0.047	0.008	0.022	11.69	11.58	0.06	0.05	1.23	1.23	10.16	52.18	0.19	5.12
72	0.046	0.007	0.021	11.44	11.34	0.06	0.05	1.20	1.20	9.95	51.07	0.18	5.01
75	0.045	0.007	0.021	11.21	11.10	0.05	0.05	1.18	1.18	9.75	50.02	0.18	4.91
78	0.044	0.007	0.020	10.99	10.89	0.05	0.05	1.16	1.15	9.55	49.04	0.18	4.81
81	0.043	0.007	0.020	10.78	10.68	0.05	0.05	1.13	1.13	9.37	48.12	0.17	4.72
84	0.042	0.007	0.019	10.59	10.49	0.05	0.04	1.11	1.11	9.20	47.25	0.17	4.63
87	0.042	0.007	0.019	10.40	10.30	0.05	0.04	1.09	1.09	9.04	46.42	0.17	4.55
90	0.041	0.007	0.019	10.23	10.13	0.05	0.04	1.08	1.07	8.89	45.63	0.16	4.48

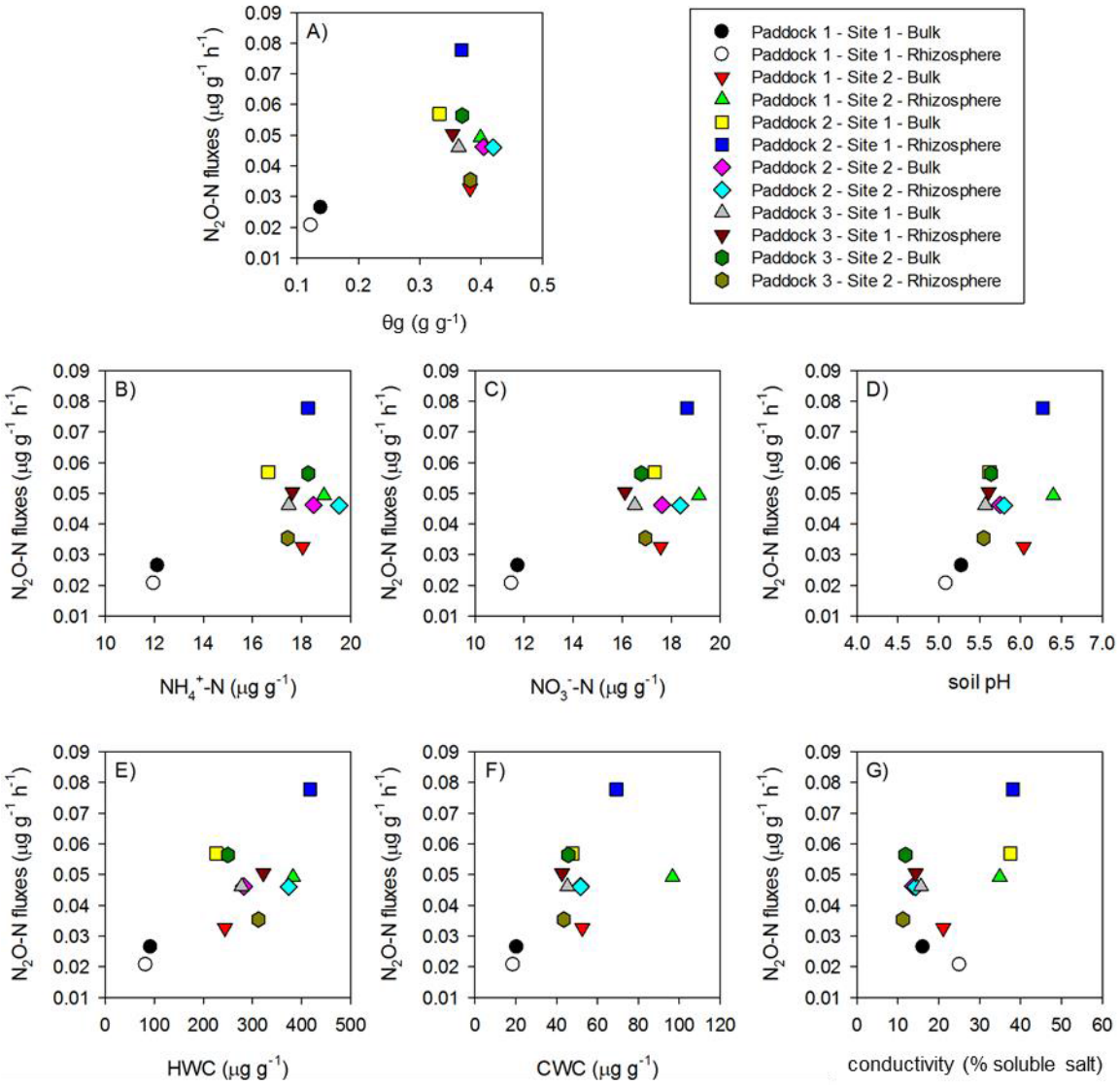
Power, 0.90 = 1-β, where β is the probability of making a type II error (failing to reject the null hypothesis when it is false). β is assume d here to be 0.10. α is assumed to be 0.10.



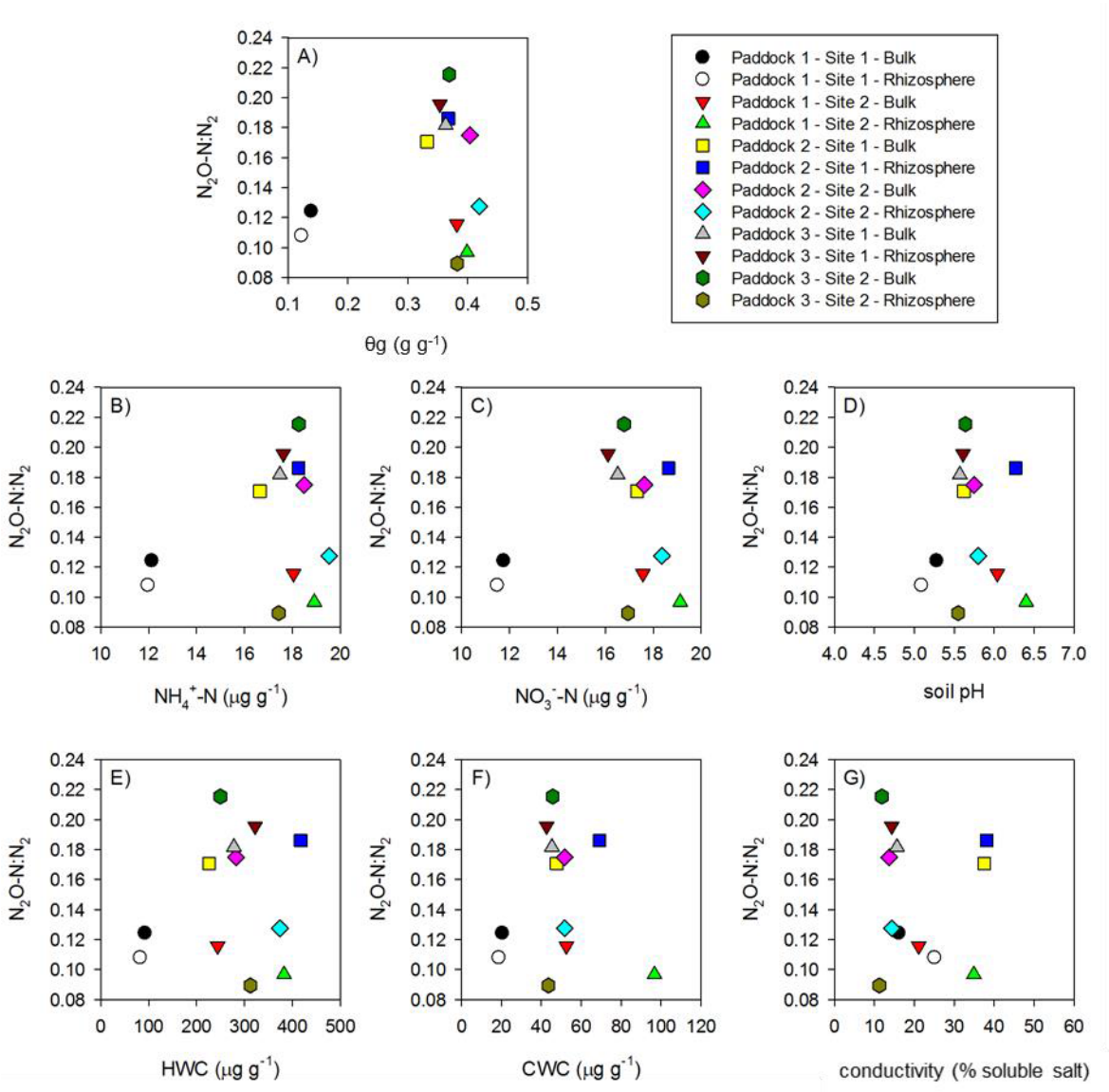
Supplementary Figure S1 Concentrations of N_2O and N_2 over the 36 hour sampling time for bulk and rhizosphere soils. Each panel represents a different paddock ("P1" representing paddock 1, "P2" representing paddock 2, "P3" representing paddock 3) and the two locations within it (S1 representing site 1 and S2 representing site 2).



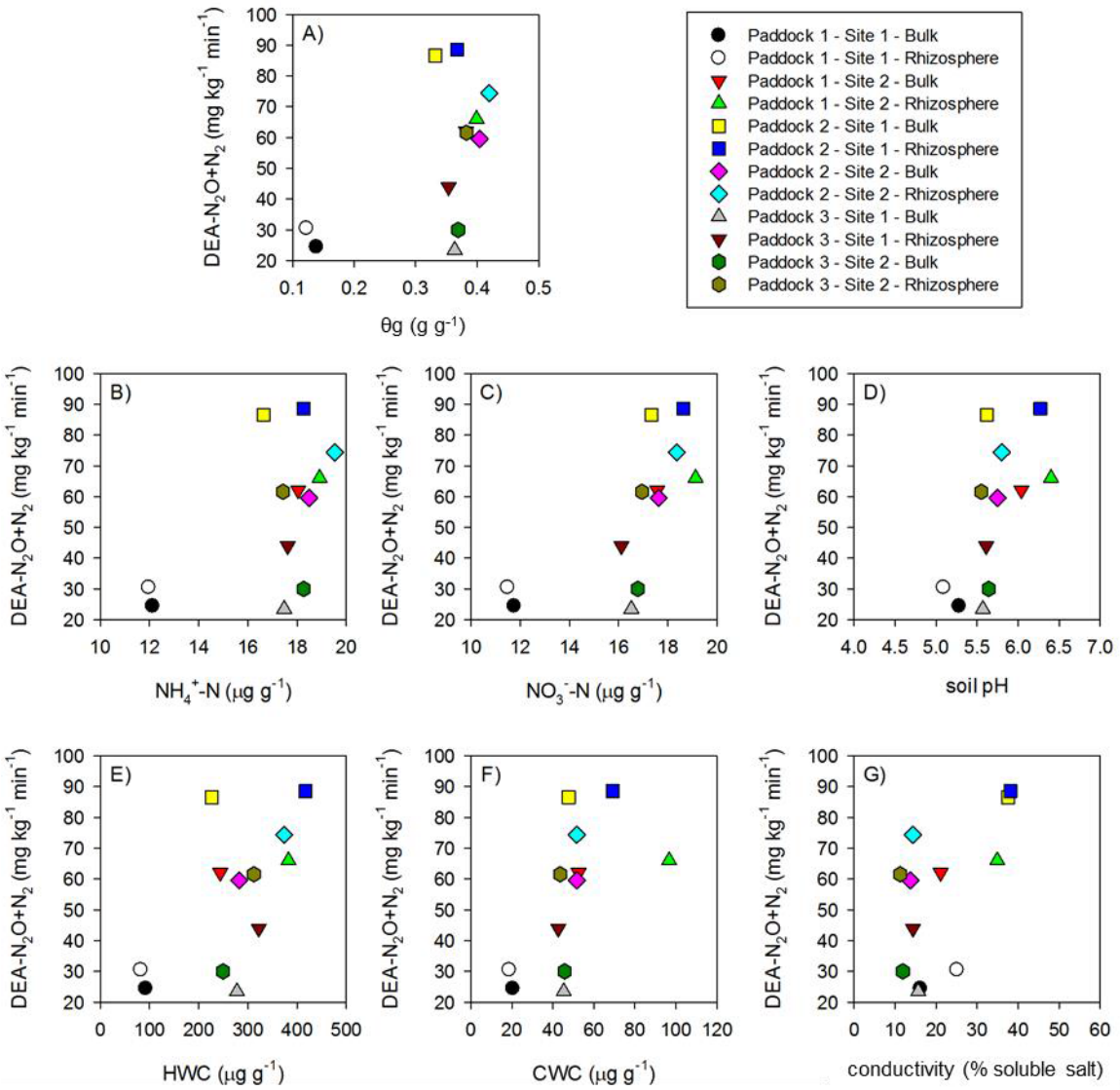
Supplementary Figure S2 Dinitrogen fluxes plotted against A) gravimetric soil moisture, B) ammonium-N, C) nitrate-N, D) soil pH, E) hot water carbon, F), cold water carbon and G) conductivity.



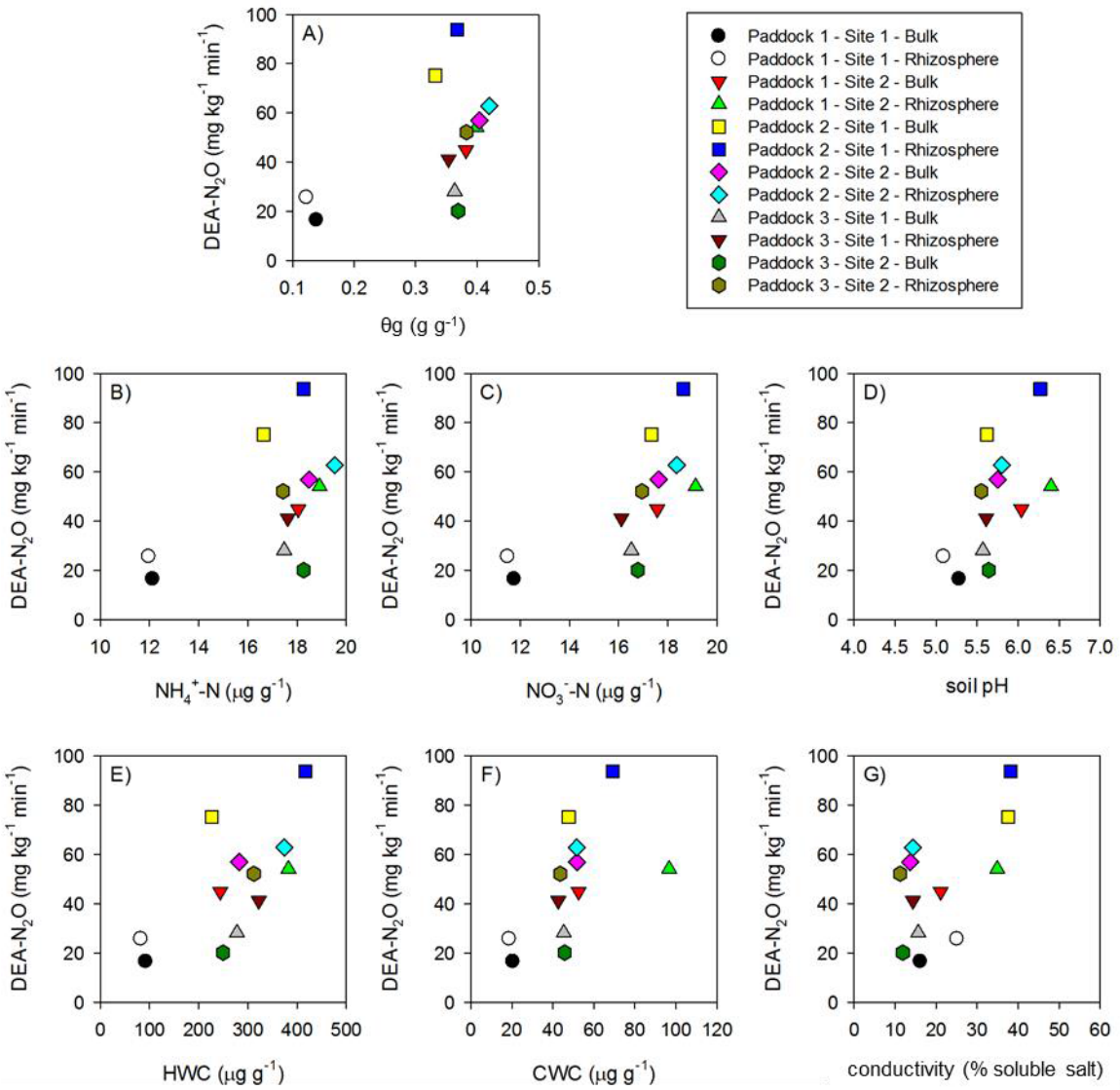
Supplementary Figure S3 Nitrous oxide fluxes plotted against A) gravimetric soil moisture, B) ammonium-N, C) nitrate-N, D) soil pH, E) hot water carbon, F), cold water carbon and G) conductivity.



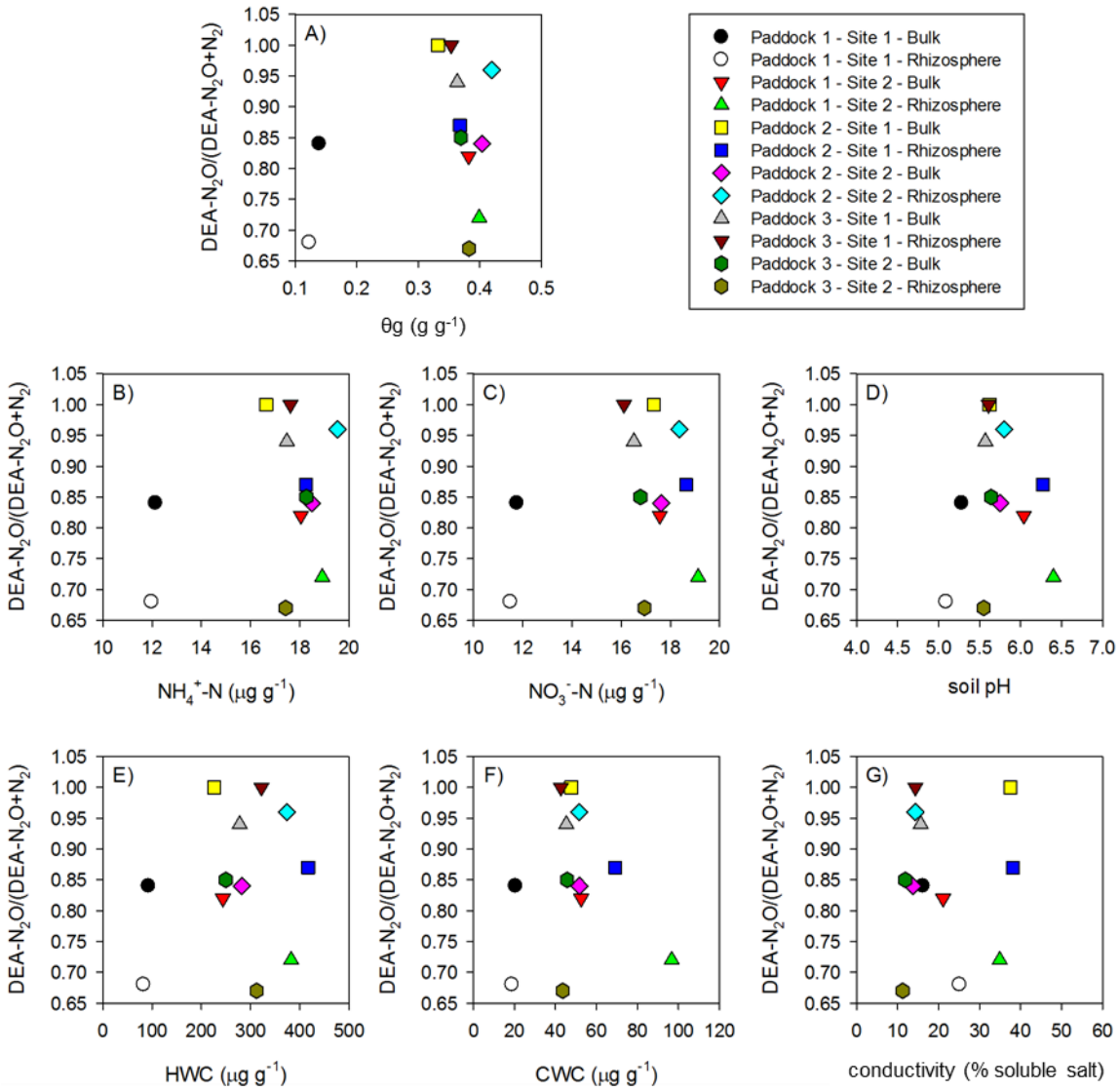
Supplementary Figure S4 The ratio of nitrous oxide and dinitrogen fluxes plotted against A) gravimetric soil moisture, B) ammonium-N, C) nitrate-N, D) soil pH, E) hot water carbon, F), cold water carbon and G) conductivity.



Supplementary Figure S5 Total nitrous oxide and dinitrogen fluxes from the denitrification enzyme assays plotted against A) gravimetric soil moisture, B) ammonium-N, C) nitrate-N, D) soil pH, E) hot water carbon, F), cold water carbon and G) conductivity.



Supplementary Figure S6 Total nitrous oxide fluxes from the denitrification enzyme assays plotted against A) gravimetric soil moisture, B) ammonium-N, C) nitrate-N, D) soil pH, E) hot water carbon, F), cold water carbon and G) conductivity.



Supplementary Figure S7 The ratio of total nitrous oxide fluxes to total nitrous oxide and dinitrogen fluxes from the denitrification enzyme assays plotted against A) gravimetric soil moisture, B) ammonium-N, C) nitrate-N, D) soil pH, E) hot water carbon, F), cold water carbon and G) conductivity.

Chapter 9. Thesis Summary, General Discussion, Conclusions, and Future Research Recommendations

9.1 Introduction

This chapter begins by discussing soil O_2 dynamics in grazed pastures. Modelled D_P/D_O and measured N_2O fluxes from the two field experiments are combined, and the potential for D_P/D_O to act as a universal predictor of N_2O occurrence in pastures is evaluated. This is followed by a discussion of the influence of soil moisture history and the presence of plants on N_2OR, and the implications for N_2O fluxes. The main conclusions of the study and recommendations for future research are listed along with their implications for working towards managing N_2O emissions from irrigated grazed pastures.

9.2 Use D_P/D_O to Explain N_2O Fluxes

Experiment 1 and Experiment 2 both showed good relationships between D_P/D_O and N_2O fluxes, and between WFPS and N_2O fluxes. When pooling the data from both experiments together, the relationship between daily average soil D_P/D_O and daily N_2O fluxes on a log-log scale shows more linearity compared to the same relationship function using θv and WFPS (Figure 9.1 b, d, and f). Of the three variables – θv, WFPS, and D_P/D_O – log D_P/D_O best explains log N_2O fluxes. When plotted on a real scale, the highest N_2O fluxes occurred over a much larger range of θv and WFPS compared to the narrow range of D_P/D_O values (Figure 9.1 a, c, and e). These results reinforce the conclusions drawn in Experiment 1 and Experiment 2; D_P/D_O is a better predictor of N_2O fluxes than θv or WFPS. Because D_P/D_O integrates porosity, bulk density and pore size distribution to better represent the soil properties compared to WFPS, it provides a reproducible explanatory variable for soil-to-atmosphere N_2O fluxes. The D_P/D_O, a physical parameter, is a good predictor of soil O_2 as dictated by soil hydrology. Soil chemical reactions that reduce soil O_2 concentrations, such as hydrology, are not captured by D_P/D_O.

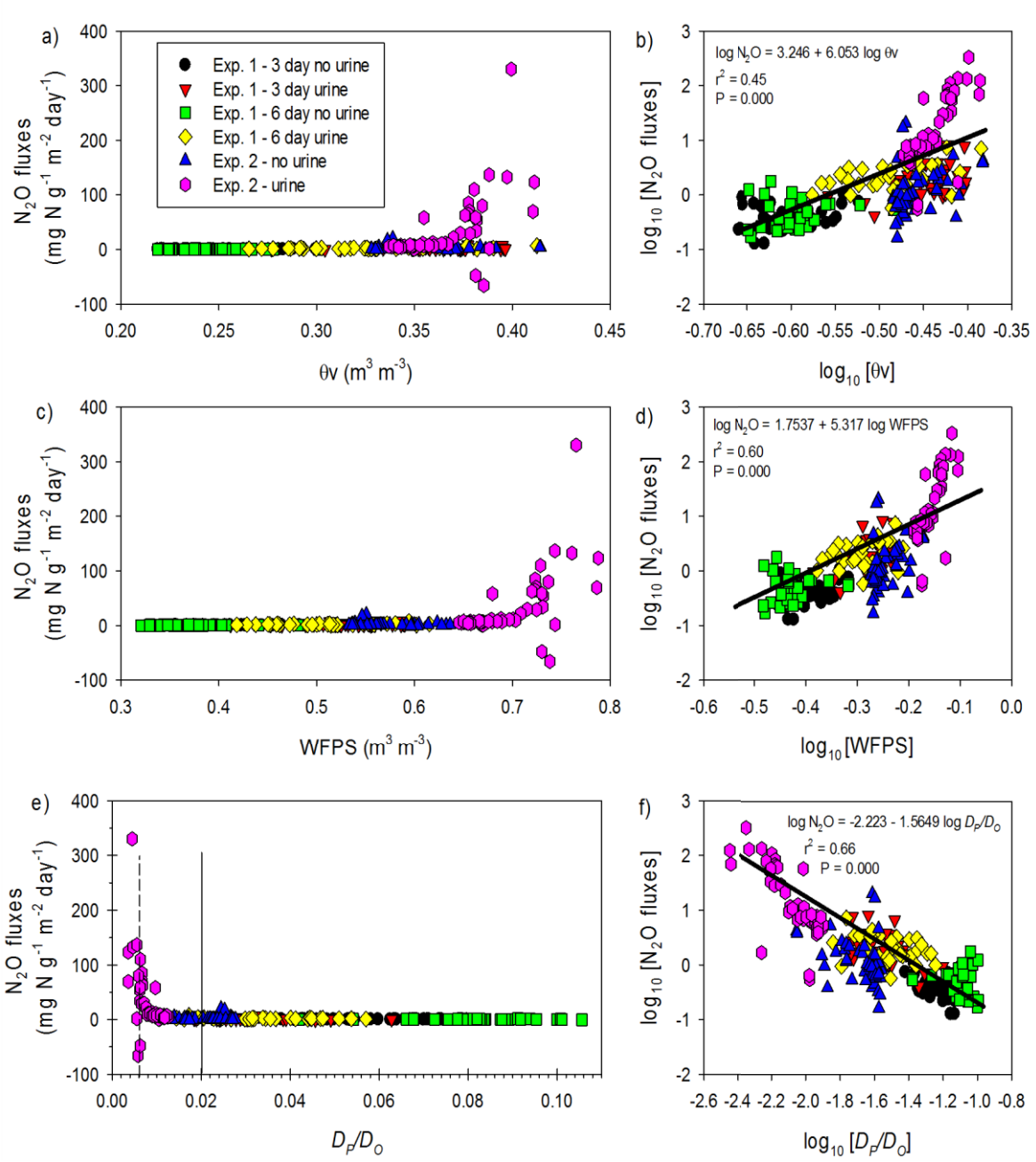


Figure 9.1 Field data from Experiment 1 and Experiment 2, presenting the untransformed and log $_{10}$ transformed N_2O and ϑv (a and b, respectively), WFPS (c and d, respectively) and D_P/D_O (e and f, respectively). The solid line on e) marks a D_P/D_O value of 0.02, the threshold identified by Stepniewski (1981) as the onset of anaerobic conditions, and the dashed line marks a D_P/D_O value of 0.006, the threshold identified by Balaine et al. (2013) as the threshold for maximum for N_2O production.

While N_2O fluxes were well explained in Experiment 1 and Experiment 2 by D_P/D_O, these experiments did not examine the relationship of D_P/D_O to other soil variables. The value of D_P/D_O is not linearly related to θv (Figure 9.2 a) or WFPS (Figure 9.2 b), with both WFPS and θv declining

exponentially with increasing D_P/D_O. This is expressed physically as a non-linear relationship between soil water content and bulk soil O_2 concentrations, which is demonstrated by relating D_P/D_O to soil O_2 concentrations. The soil O_2 at 50 (Figure 9.2 c) and 100 mm depths (Figure 9.2 d) decrease only when D_P/D_O approaches the threshold of 0.02, which was noted in Stepniewski (1981) as the threshold for anaerobic conditions. Thus, soil bulk O_2 does not decrease at all depths when soil moisture increases. There were greater decreases in soil O_2 at low D_P/D_O values at 100 mm soil depth compared to 50 mm soil depth (Figure 9.2 c, d). This is consistent with obstruction of O_2 transport into the soil profile by soil water content, and reduced by microbial respiration, leading to greater O_2 extinction with greater soil depth (Cook et al., 2013).

Future studies could readily build on this work by modelling D_P/D_O as was done in Experiment 1 and Experiment 2. Modelling D_P/D_O, according to Moldrup et al. (2013a), requires θv and bulk density, which can be used to derive air-filled porosity and total porosity. Any study reporting WFPS, which is calculated by dividing the volume of water in the soil by the total porosity (Linn and Doran 1984) can use this information to also calculate D_P/D_O according to the model in Moldrup et al. (2013a).

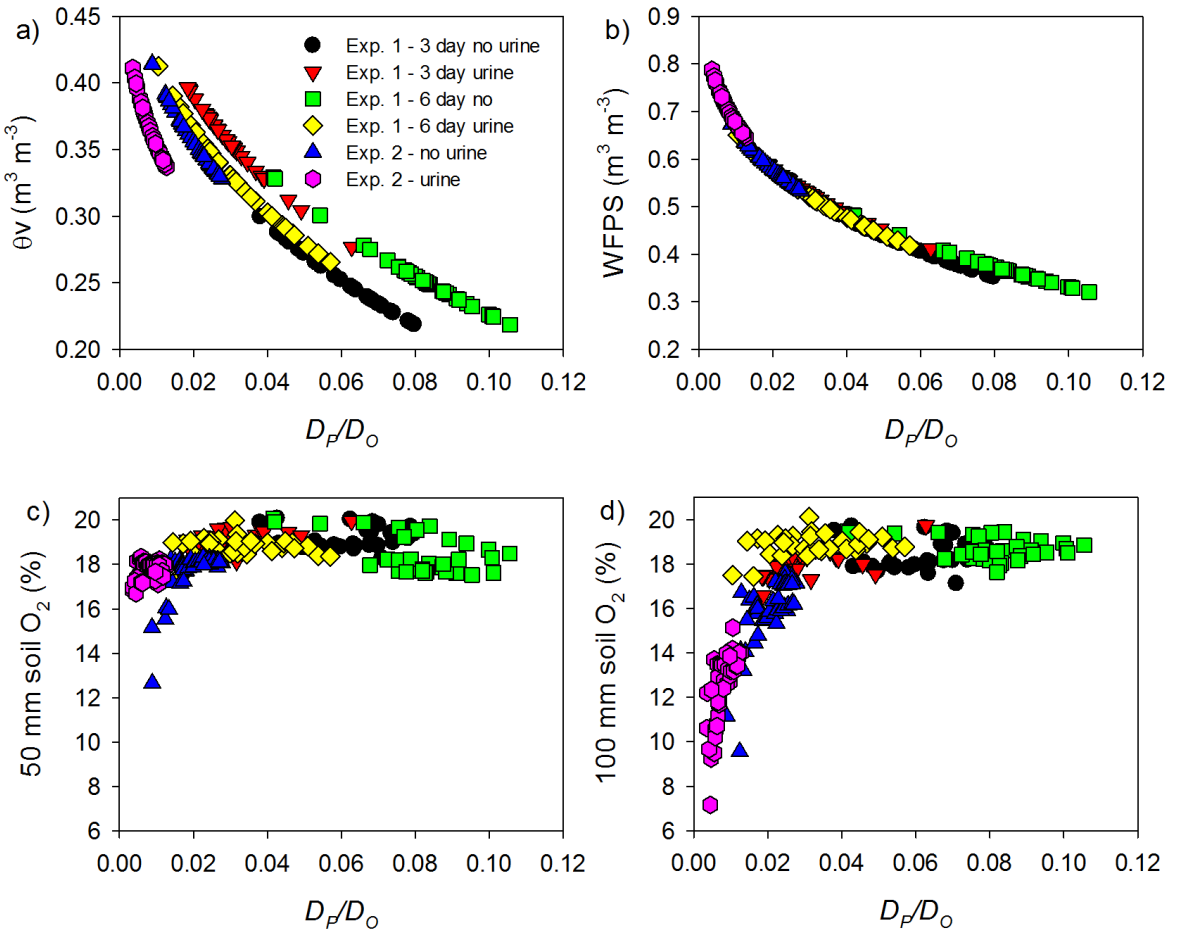


Figure 9.2 The relative soil gas diffusivity (D_P/D_O) data from Experiments 1 and 2 plotted against a) volumetric water content and b) water-filled pore space, c) soil oxygen at 50 mm soil depth, and d) soil oxygen at 100 mm soil depth

9.3 Diel Cycling of Soil O_2 in Pasture

The potential for diel cycling of N_2OR was assessed in Experiment 4, but that experiment was focused on constraining the effects of diel cycling of root exudates on N_2OR. Experiment 1 and Experiment 2 are the first intensive measurements of soil O_2 made in grazed pasture soils. These measurements suggest the diel cycling of soil O_2 is related to plant activity. To show the diel cycling of soil O_2 is related to respiration, soil CO_2 concentrations (GMP 222, Vaisala, Helsinki, Finland) were measured at 10 mm soil depth in both the urine and no urine treatments during Experiment 2. The CO_2 sensors were covered with Tyvek HomeWrap® (DuPont, USA), which is permeable to gas but not to water, to prevent environmental damage to the sensor.

Soil CO_2 concentrations and soil O_2 concentrations in the back paddock at Lincoln University co-varied (Figure 9.3 a). Diel cycling of soil O_2 had an amplitude of 1-2% over 24 h and was inversely related to solar radiation (Figure 9.3 c). Soil O_2 was also inversely related to, but lagged, soil temperature (Figure 9.3 b).

Photosynthesis driven diel O_2 cycling in soil contributes to the creation of anaerobic microsites under non-saturated soil conditions (Müller and Sherlock 2004). Given that decreased soil O_2 increases N_2OR production, the response of N_2OR to diel changes in soil O_2 should be a focus of future studies because it may influence diel N_2O dynamics (Christensen 1983, Smith et al. 1998, Denmead 2008). Studies making use of molecular tools such as ribonucleic acid (RNA) sequencing analysis, which shows functional activity in soil microbes, would be of use in pursuing such an objective.

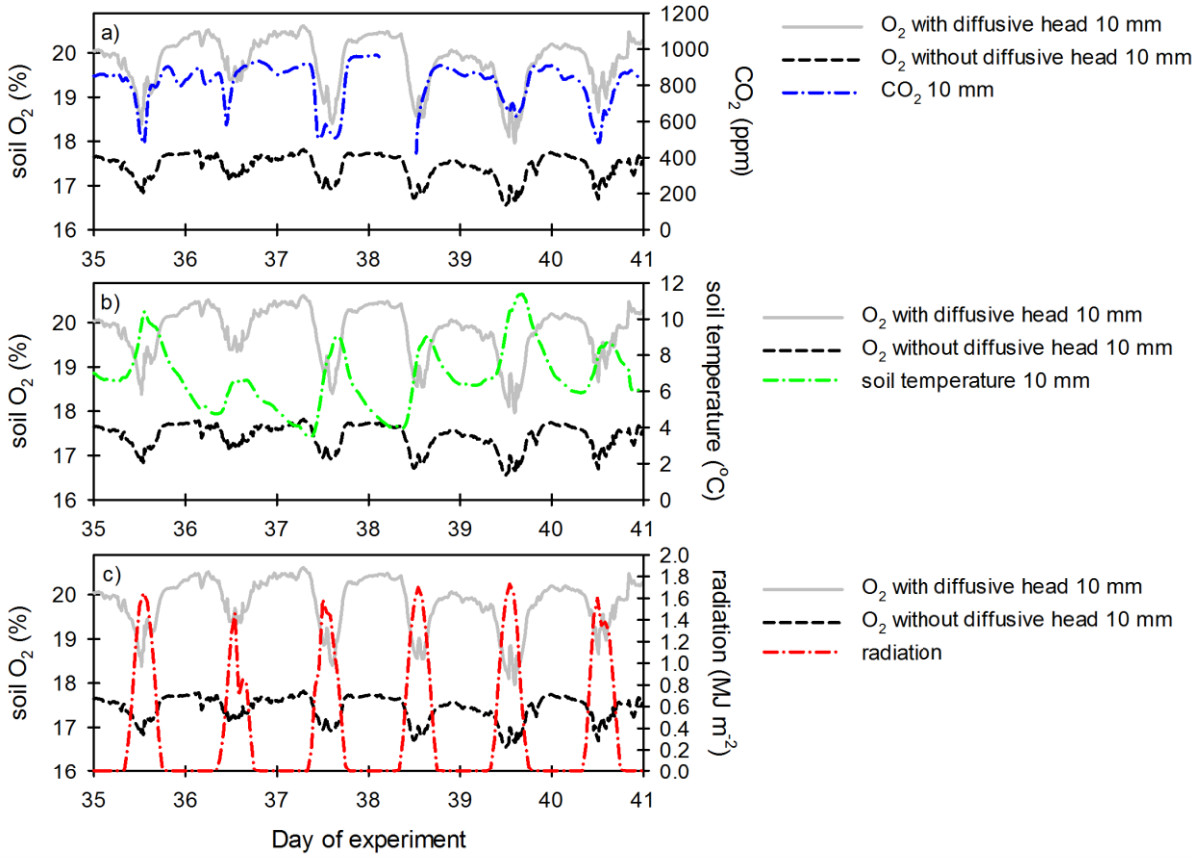


Figure 9.3 Soil oxygen (O_2) from the urine treated soil from both the sensor with and without the diffusive head plotted against a) carbon dioxide (CO_2) concentrations, b) soil temperature, and c) radiation.

9.4 Episodic Changes to Soil O_2

Experiment 1 and Experiment 2 show for the first time the soil O_2 concentrations under a urine patch decrease for ≈ 24 h after urine deposition. This suggests N_2O pulses after urine deposition may be due to denitrification using indigenous NO_3^- available within the soil. The decrease in soil O_2 concentration is due the urea embodied within urine and the ensuring hydrolysis reactions, and not the additional water added by the urine. Immediately after urine deposition, the soil D_P/D_O value does not decrease below the 0.02 threshold needed to trigger a decrease in soil O_2 concentrations, which were observed when soil moisture increased (Chapter 4 and Chapter 5). However, soil O_2 still decreases, due to production of CO_2 during hydrolysis reactions.

After urine deposition, decreases in soil O_2 concentrations are greater with deeper soil depth. Future research could explore this finding by examining how soil bulk density influences the magnitude of soil O_2 concentration decrease after urine deposition. Urine deposited onto more compact soil may result in more concentrated band of urine compared to uncompact soils. This may lead to lower soil O_2 concentrations in the soil profile of a more compact soil due to more concentrated zones of respiration and urea hydrolysis. Because of the lower total porosity, this may also mean less voids in the soil for O_2 to fill, which may cause the soil to become more anaerobic sooner, or for longer.

9.5 Antecedent Moisture Conditions and N_2O Fluxes

Experiment 3 showed that potential N_2OR, derived from denitrification enzyme assays (DEA's), changed over time after a wetting event. This occurred despite maintaining consistent matric potential, which was achieved by keeping the soil cores on tension tables after the wetting event. This showed that N_2OR was greatly affected not only by changes to soil moisture and soil O_2, but also by biological changes (microbial activity), and soil chemistry and substrate availability. These factors need to be considered in tandem with changes to soil moisture and soil O_2 when considering how soil moisture history influences potential N_2OR. More studies are needed to constrain how individual soil chemical factors, e.g. pH, nitrate, carbon in various forms (simple vs complex), and different permutations of these factors, influence N_2OR activity independent of changes to soil moisture and soil O_2. Doing so will help to interpret the effects of soil moisture history on N_2O:N_2 ratios.

Along with the soil chemistry and biological activity, future work should consider integrating D_P/D_O into experiments examining the effects of soil moisture history on N_2O fluxes and N_2OR along with soil moisture or soil O_2. The N_2OR pathway is primed by a history of high soil moisture due to decreased soil O_2, thereby affecting the N_2O:N_2 ratio (Uchida et al. 2014). However, as was shown in the previous section (9.2), for bulk soil O_2 to decrease, D_P/D_O must reduce to 0.02 or less (Figure 9.2 c, d). This infers that D_P/D_O can help increase our understanding of when the denitrification product ratio will change due to antecedent moisture conditions because it helps us to understand when soil O_2 supply into the bulk soil will decrease. For example, a precipitation event on a compact, poorly drained soil may reduce D_P/D_O below the 0.02 threshold. This would prime the N_2OR pathway. A proceeding precipitation event may experience lower N_2O fluxes due to greater reduction of N_2O to N_2 since the N_2OR pathway was already primed due to a recent history of soil O_2 depletion. However, a similar precipitation event on a loose, freely draining soil may not reduce the value of D_P/D_O, and therefore have little, if any, influence on N_2OR, and the denitrification product ratio.

9.6 The Effect of Plants on N_2O Fluxes and N_2OR: Issues and Recommendations

In Experiment 3, as with many laboratory experiments, plants were excluded to obtain greater control over the soil conditions. As a part of Experiment 4, the N_2O:N_2 ratio, N_2O fluxes, and CO_2 fluxes, and denitrification potential, were compared in soils with and without plants. The results suggested that the presence of plants increased the denitrification potential and CO_2 fluxes. It was expected that the greater respiration rates in the soils with plants would decrease soil O_2 in addition to moisture driven decreases in soil O_2. As a consequence, there would be lower N_2O:N_2 ratios in the soils with plants, compared to those without plants. Emissions of N_2O and N_2 were expected to be higher in general from the planted soil, because along with greater denitrification potential, the rhizosphere would increase the soil C supply, thereby increasing denitrification rates. However, the plants in the repacked soil cores changed the soil structure compared to the repacked soil cores without plants, leading to D_P/D_O values and soil O_2 concentrations that differed between treatments at the same soil moisture contents in unexpected ways. Introducing plants to laboratory studies needs to be done more often because of the clear influence plants have on the outcomes of experiments. However, how plants influence soil structure should be considered in the development of future studies that aim to compare planted and unplanted soils. A water

retention curve, for example, could help infer how soil moisture-mediated O_2 diffusion differs between soils with and without plants.

Pooling the DEA results from the experiments, both laboratory and *in situ*, showed that mean DEA-N_2O+N_2 and mean DEA-N_2O were higher in planted soils compared to soils without plants, suggesting that plants increase the denitrifying capability of soils. However, the mean DEA-N_2O/(DEA-N_2O+N_2) is lower in plants compared to no plants, suggesting plants reduce the N_2OR activity in soil. A possible explanation for this is that the presence of plants influence soil structure, allowing for greater diffusion of O_2 into the soil, thus inhibiting N_2OR as discussed in the previous paragraph. Despite the presence of plants increasing soil denitrification potential, there was no statistical difference in N_2O fluxes or denitrification potential between the rhizosphere and bulk soil when tested in Experiment 5, Chapter 5. This may be due to ineffective separation of the soils, too few samples, or high spatial variability. More research is needed to better understand how the rhizosphere influences the soil environment, and on what scale this influence is important.

Pooling of all of the data, the DEA showed the presence of plants resulted in higher NH_4^+-N, NO_3^--N, and pH. This is consistent with the effects that the rhizosphere is expected to have on the soil (Lynch and Whipps 1990, Cheng et al. 2003, Philippot et al. 2009, Zhu et al. 2014). There was also higher HWC in the soil with plants, which is indicative of higher microbial biomass (Ghani et al. 2003), consistent with previous literature that suggests plants increase soil microbes (Rovira 1965, Foster 1988, Smalla et al. 2001).

A combination of factors will interact to affect potential N_2OR. A backwards stepwise regression using θv, NH_4^+-N, NO_3^--N, CWC, HWC, and pH shows that NH_4^+-N and CWC explain 50.32% (adjusted R^2) of the variability in DEA-N_2O/(DEA-N_2O+N_2). The regression equation can be expressed as:

$$DEA\text{-}N_2O/(DEA\text{-}N_2O+N_2) = 0.8425 + 0.001143\ NH_4^+\text{-}N - 0.001925\ CWC$$

The relatively low mean θg (measured before the soil was used in the DEA as a slurry), suggests nitrification was generally the dominant process before the enzyme assays, hence the significant influence of NH_4^+. The CWC is considered to be leachable C (Ghani et al. 2003), however, there was no difference in CWC concentrations between the planted and unplanted soil (Table 9.1), despite its apparent influence on potential N_2OR. Perhaps there is a particular form of C captured by the CWC extraction that influences denitrification potential and N_2OR potential. This should examined in future research.

Table 9.1 The mean and standard error (± SEM) for the denitrification enzyme assays (DEA) and soil enviornmental data.

	plants		no plants		P-value
Variable	mean	± SEM	mean	± SEM	
DEA-N_2O+N_2 (mg N kg^{-1} min)	45.66	3.43	29.60	4.65	0.010
DEA-N_2O (mg N kg^{-1} min)	31.28	3.46	12.50	2.11	0.000
DEA-N_2O/(DEA-N_2O+N_2)	0.69	0.05	0.48	0.07	0.020
Gravimetric soil moisture	0.34	0.01	0.38	0.02	0.094
Ammonium(µg N g^{-1})	78.70	26.10	3.55	0.73	0.007
Nitrate (µg N g^{-1})	66.3	15.3	26.39	4.32	0.017
Cold water carbon (µg g^{-1})	137.4	22.5	166.7	21.6	0.354
Hot water carbon (µg g^{-1})	1951	264	982.9	91.9	0.001
Soil pH	5.80	0.09	5.53	0.09	0.039

9.7 Conclusions and Implications for Irrigation use in the New Zaland Dairy Industry

This thesis set out to explore how irrigation influenced N_2O fluxes and N_2OR from grazed pasture soil. The following conclusions can be drawn:

1) **Bulk soil O_2 will decrease when D_P/D_O approaches 0.02**. Therefore D_P/D_O might be useful for developing strategies that manipulate O_2 to 1) keep bulk soil aerobic to therefore keep N_2O fluxes negligible, or 2) reduce soil O_2 to encourage N_2OR and reduce N_2O to N_2. How irrigation influences D_P/D_O will be influenced by soil type and properties, irrigation intensity, and irrigation frequency. However, D_P/D_O does not capture decreases in soil O_2 associated with urine deposition because the reduction in soil O_2 is related to chemical reactions and not increases in soil moisture.

2) **The D_P/D_O variable provides a reproducible threshold for high N_2O fluxes**. At the threshold of D_P/D_O 0.006, N_2O fluxes reach a maximum when there is substrate available for denitrification or nitrifier-denitrification, and may show evidence of N_2O uptake. This occurs as soil conditions become reducing enough for N_2O production via denitrification and nitrifier-denitrification, and for N_2OR production to result in N_2 production. The work in this thesis builds on earlier laboratory studies performed under controlled conditions with repacked soil cores (Balaine et al. 2013, Balaine et al. 2016). The results from this thesis support approximately the same threshold for N_2O and N_2 production in the field as

has been previously found (Balaine et al. 2013, Balaine et al. 2016). However, because this thesis is only comprised of two field based datasets on two different soil types, more data are needed to further test the validity of these thresholds *in situ*. Importantly, however, these field datasets show that over a range of bulk densities, D_P/D_O better explained to N_2O fluxes when compared to WFPS.

3) **Antecedent moisture conditions influence N_2OR.** The activity of N_2OR is greater closer to a wetting event due to higher soil moisture, thus it was found that there is greater potential for N_2OR activity in a more frequently irrigated soil. However, changes to soil chemistry and soil biology also change with soil moisture history, and must be considered along with soil O_2 and soil moisture changes when understanding how N_2OR behaves under different irrigation regimes.

4) **Future irrigation studies should include plants if conducted in the laboratory because plants influence experimental outcomes.** The effects of plants on N_2O fluxes and denitrification potential differ based on whether or not a planted soil is compared to a soil without plants, compared to a planted soil where the bulk and rhizosphere soil are separated. How plants are incorporated into the experiments (planting sieved soil vs. using intact soil cores) will also affect the soil chemistry and soil structure, which will influence the experimental outcomes. These factors need to be considered during the experimental design process.

While the information from this thesis is helpful for understanding the dynamics of N_2O fluxes from irrigated systems, the results cannot be directly extrapolated for use by the New Zealand dairy industry to improve farm management practices without more information. This is because further comprehensive consideration of related factors is required. These include a water balance to compensate for water use by the plants and soil leaching, seasonal dynamics in climate, which will affect the soil moisture as precipitation changes seasonally as does photosynthesis rates. In turn, these seasonal factors will influence the soil O_2 concentrations, which will influence N_2O fluxes and N_2OR functioning.

9.7.1 Summary of Future Research Requirements

The work completed in this thesis has raised some questions for future work. Research is needed to explore D_P/D_O and soil O_2 in various soil types and under different climatic and moisture conditions. Understanding the potential interactions between D_P/D_O and different permutations of environmental conditions such as substrate supply and pH, and respiration rates is important since it will influence soil O_2 supply and may modify the D_P/D_O thresholds for N_2O and N_2OR identified in the thesis. This may be especially important for informing how micro-pore O_2 dynamics in aerated soil, influence N_2O and N_2OR. This could also help understand variation of N_2O and N_2OR on various temporal scales (i.e. seasonal, diel, etc.).

For a better quantification of the relationships between N_2O and N_2OR, and D_P/D_O, future work should consider using ^{15}N isotopes for partitioning N_2O/N_2 ratios, along with direct measurements of N_2OR using molecular techniques, and link the results to D_P/D_O. Use of these advanced molecular or genetic techniques will help inform the effects of antecedent soil moisture on N_2OR. In order to effectively interpret how soil moisture/soil O_2 history influence N_2OR, different combinations and permutations of environmental factors, such as pH, on N_2OR ratios, from different soil moisture histories, is required. This would help to understand how these conditions influence the repression and de-repression of N_2OR in combination with O_2 histories and concentrations.

This thesis noted high spatial variability. This variability has implications for the extrapolation of local results to a field scale. The link between D_P/D_O and trampling, and how this influences the ensuing N_2O and N_2 fluxes from the soil from different paddocks, should be explored in future studies, as this may help explain some spatial variability. The spatial variability may also be attributed to the effect of fertilizer history, grazing history, or inherent natural variability, on N_2O fluxes and N_2OR, and denitrification potential, or inherent natural variability, which may have nothing to do with land management. These factors should be considered in future research. Future research that does not composite samples should include many samples to improve confidence in statistical analysis.

Insight into this spatial variability may be partially explained by a better understanding of the effects of plants on N_2O fluxes and N_2OR are required for effectively transferring lab results to the field. To better understand diel N_2OR dynamics, future research should consider measuring changes in root exudate composition and concentrations from pasture plants over the course of

the day, as this may influence N_2O and N_2OR, since different forms of C in combination with difference O_2 concentrations affect NO_3^- reduction differently. This thesis found that there were similarities between bulk and rhizosphere soil for all measured factors, likely due to separation of rhizosphere and bulk soil. Methods for more precise separation of bulk and rhizosphere soil are required to decipher the sphere of influence for the rhizosphere in soil.

9.8 References

Balaine, N., T. Clough, M. Beare, S. Thomas, and E. Meenken. 2016. Relative gas diffusivity controls on soil nitrous oxide and dinitrogen fluxes and their ratios. Soil Science Society of America. DOI: 10.2136/sssaj2015.09.0350

Balaine, N., T. Clough, M. Beare, S. Thomas, E. Meenken, and J. Ross. 2013. Changes in Relative Gas Diffusivity Explain Soil Nitrous Oxide Flux Dynamics. Soil Science Society of America Journal 77:1496-1505.

Cheng, W., D. Johnson, and S. Fu. 2003. Rhizosphere effects of decomposition - controls of plant species, phenology and fertilization. Soil Science Society of America Journal 67:1418-1427.

Christensen, S. 1983. Nitrous oxide emission from a soil under permanent grass: Seasonal and diurnal fluctuations as influenced by manuring and fertilization. Soil Biology and Biochemistry 15:531-536.

Cook F. J., J. H. Knight, and F. M. Kelliher. 2013. Modelling oxygen transport in soil with plant root and microbial oxygen consumption: Depth of oxygen penetration. Soil Research. 51:539–553

Denmead, O. T. 2008. Approaches to measuring fluxes of methane and nitrous oxide between landscapes and the atmosphere. Plant and Soil 309:5-24.

Foster, R. C. 1988. Microenvironments of soil microorganisms. Biology and Fertility of Soils 6:189-203.

Ghani, A., M. Dexter, and K. W. Perrott. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology and Biochemistry 35:1231-1243.

Linn, D. M., and J. W. Doran. 1984. Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. Soil Science Society of America Journal 48:1267-1272.

Lynch, J. M., and J. M. Whipps. 1990. Substrate flow in the rhizosphere. Plant and Soil 129:1-10.

Moldrup, P., T. K. K. Chamindu Deepagoda, S. Hamamoto, T. Komatsu, K. Kawamoto, D. E. Rolston, and L. Wollesen de Jonge. 2013. Structure-Dependent Water-Induced Linear Reduction Model for Predicting Gas Diffusivity and Tortuosity in Repacked and Intact Soil. Vadose Zone Journal 12:2-11.

Müller, C., and R. Sherlock. 2004. Nitrous oxide emissions from temperate grassland ecosystems in the Northern and Southern Hemispheres. Global Biogeochemical Cycles 18:GB1045.

Philippot, L., S. Hallin, G. Börjesson, and E. M. Baggs. 2009. Biochemical cycling in the rhizosphere having an impact on global change. Plant and Soil 321:61-80.

Rovira, A. D. 1965. Interactions Between Plant Roots and Soil Microorganisms. Annual Review of Microbiology 19:241-266.

Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, and G. Berg. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology 67:4742-4751.

Smith, K. A., P. E. Thomson, H. Clayton, I. P. McTaggart, and F. Conen. 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmospheric Environment 32:3301-3309.

Stepniewski, W. 1981. Oxygen diffusion and the strength as related to soil compaction. II Oxygen diffusion coefficient. Polish Journal of Soil Science 14:3-13.

Uchida, Y., Y. Wang, H. Akiyama, Y. Nakajima, and M. Hayatsu. 2014. Expression of denitrification genes in response to a waterlogging event in a Fluvisol and its relationship with large nitrous oxide pulses. FEMS Microbiology Ecology 88:407-423.

Zhu, B., J. Gutknecht, D. Herman, D. Keck, M. Firestone, and W. Cheng. 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology and Biochemistry 76:183-192.